Antibacterial Activity of Twelve Medicinal Plants

Thesis submitted to Uinversity of Khartoum for the Degree of Doctor of Philosophy of Pharmaceutical Microbiology

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Dedication

To the souls of my late parents, Abdelmageed and Nafeesa, and my late older sister Fatimam and late brother Ahmed Albadawi.

To the younger generations of my family, hoping that this work will inspire them to ex cel in their lives.

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مستخلص

العنوان: فاعلية اثنى عشر نبات طبي لمقاومة البكتيريا

مقدمة:

مقاومة البكتيريا المتصاعدة لكل من المضادات الحيوية التقليدية والبديلة جعلت من الصعب معالجة أمر الجروح الملتهبة والخراجات والأمراض الخمجية لذا كان هدف هذه الدراسة هو إيجاد مضادات حيوية مستخلصة من النباتات الطبية التي استخدمت لآلاف السنيين في الطب الشعبي السوداني.

الأهداف:

صممت هذه الدراسة لمعرفة حساسية خمسة أنواع بكتيرية معزول من الجروح والخراجات ضد مستخلصات اثنى عشر نبات طبي والانواع البكتيرية هي الأشريشية القولونية، العنقودية الذهبية ، الزائفة الزنجارية ، الكبسيلا الرئوية والمتقلبة الرائعة أما الخلاصات فقد أخذت من النباتات الطبية الآتية المريمية قشر الرمان، لب الرمان، الثويا (العفصة)، حب العروس، الذريدة، الحنوة، اليسر (الرواق) ، الكرمدودة الحلييت، الفكهت، عرق الحجر والصحب.

المواد وطرق البحث:

تقييم حساسية الأنواع البكتيرية المختلفة تجاه مستخلصات النباتات الطبية المختارة يعتمد على وجود حيز من التثبيط وقياس محيطة وذلك اعتماداً على وسيلة الانتشار في الآجار.

حتى تتم المقارنة تم استعمال كل من البكتيريا الاختيارية الأشريتية القولونية (ATCC25 922) العنقودية الذهبية (ATCC25923) الزائفة الزنجارية (ATCC

27853) ، كليبسيلا الرئوية (ATCC35657) والعضوية (NCTC8236) وأيضاً استعملت المضادات الحيوية التقلديةي الأتية بنزايل ينسلين، ايرثرومايسين، جينتامايسين، وسيفترزون .

النتائج:

باستعمال طرق البحث الاختيارية الكيفية تم التعرف على المكونات النباتية الكبيرة لخلاصات كل من الأثنى عشر نبات طبي وقد تم ايجاد الأعداد والكميات المختلفة لكل من العصيات الفلافنيدات الصابونيات، جليكوسليدات القلبية، جليكوسيدات الفينولية ، جليكوسيدات الأنثركونية، جليكوسيدات السيانوجينية، والقلويدات.

تم عزل مئة نوع بكتيري بواسطة الوسائل الاختبارية ووجد على أنها تتكون من بكتيريا سالبة الجرام في الاشريشية القولونية (20%) الزائفة الزنجارية (20%) المتقلبة الرائعة (18%) كليسيلا الرئوية (17%). وبكتيريا موجبة الجرام هي العنقودية الذهبية (18%) والعنقودية الذهبية المقاومة للميثسلن (7%) وهي نسبة من الانتشار يجب الانتباه لها.

معظم خلاصات النباتات الطبية التي استخلصت بواسطة الميثانول أظهرت حساسية عالية ضد كل عن الاشريشية القولونية والزائفة الزنجارية والمتقلبة الرائعة وكلسيلا الرئوية والعنقودية الذهبية وقد وجد على أنها أقوى من تلك التي الخلاصات التي استخلصت بواسطة البنزين والكلورفورم.

كل خلاصات النباتات الطبية المختبرة أظهرت حساسية ضد العنقودية الذهبية المقاومة للميثسلن كما أنه وجد على أن قشر الرمان يحتوي أقوى المضادات البكتيرية فاعلية ويليه التويا (العفصة) ثم الصحب.

الخلاصة:

عند اختبار خلاصة الثويا (العفصة) بوسيلة التخفيف أظهرت أقل تركيز مثبط ضد كل الأنواع البكتيرية الاختيارية، لذا تعتبر هذه الخلاصة أقوى مضاد بكتيري واسع المدى بينما أظهرت خلاصة قشر الرمان أعلى تركيز مثبط للبكتيريا الاختيارية وقد تمت ملاحظة أن البكتيريا العصوية هي الأكثر استجابة لخلاصات كل من الصحب ، قشر الرمان ، الثويا.

الفاعلية الجيدة للمضادات البكتيرية لخلاصات كل من قشر الرمان والثويا والصحب ضد العنقودية الذهبية المقاومة للميثسلن أبرزت قوتها كمصدر لتحسين العلاج وإمكانية اجتثاثها لذا وجب إجراء المزيد من الاختبارات عليها.

هذه الدراسة أوضحت مدى الحوجة لمزيد من الاختبارات على خلاصات الميثانول والتي تحديداً ظهرت كمضادات بكتيرية جيدة الفاعلية وأيضاً الحوجة لإجراء اختبارات على خلاصات البنزين والكورفورم للنباتات الطبية المختبرة لاكتشاف مدى فاعليتها.

Abstract

The ever growing bacterial resistance to both conventional and alternative antibiotics made it very difficult to manage infected wounds and abscesses and other infectious diseases. Hence, the aim of this study was to find antibacterial agents from a collection of medicinal plants that have been in use for thousands of years in Sudanese traditional medicine.

This study was designed to evaluate the susceptibility of 5 bacterial species isolated from wounds and abscesses to extracts of 12 medicinal plants. The bacterial isolates were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella sp* and *Proteus vulgaris*; and the extracts were taken from *Salvia orientalis, Punicar granatum* (Bark and Wood), Biota *orientalis, Piper cubeba, Acorus calamus, Candula arvensis, Moringa perigrina, Nauclea lotifolia, Ferula asafoetida, Cissus petiolata,* and *Petasites hybridus*.

The evaluation of bacterial susceptibility to the selected medicinal plants was based on the presence or absence of inhibition zone and zone diameter, using the cup plate agar diffusion method.

For comparative reasons standard *Escherichia coli* (NCTC 25922); *Staphylococcus aureus* (NCTC 25923); *Pseudomonas aeruginosa* (NCTC 27853); *Klebsella sp* (NCTC 35657); *Bacillus subtilis* (ATCC 8236), and conventional antibiotics including Benzylepenicillin, Erythromycin, Gentamycin and Ceftriaxone were used

The major phytoconstituents in the extracts of the 12 medicinal plants were identified using standard qualitative methods. Varying numbers

and quantities of Tannins, Flavonoids, Saponins, Cardic glycoside, phenolic glycoside, Anthraquinone glycoside, Cyanogenic glycosides and Alkaloids were found.

Adopting the standard methods, 100 bacterial organisms were isolated, and found to consist of Gram's negative bacteria (*Escherichia coli* (20%); *Pseudomonas aeruginosa* (20%); *Proteus vulgaris* (18%) and Klebseilla sp. (17%); and Gram's positive *Staphylococcus aureus* (18%); and 7% of Methicillin-resistant-*Staphylococcus aureus* (MRSA) which could be concidere as an alarming level of prevalence.

Methanolic extracts of medicinal plants against bacterial isolates, high sensitivity was shown by: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella sp* and *Staphylococcus aureus* to the majority of the test medicinal plants. This sensitivity was found to be more than that of the benzenic and chloroformic extracts.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has shown sensitivity to all tested medicinal plants extracts, however, *Punicar granatum* (Bark) gave the highest antibacterial activity followed by *Biota orientalis* and then *Anogeissus leiocarpus.* These results revealed the potentials of these plants as a source of effective antibacterial agents that can improve the treatment and possibly the eradication of MRSA

***Biota orientalis* extracts when tested using agar plate dilution method, have shown the lowest MIC against all tested standard bacteria, hence it was considered as a potential wide-spectrum antibacterial medicinal plant extract. Whereas *Punicar granatum (Bark*) showed that it has the highest MIC. Also, it was observed that *Bacillus subtilis* is the most**

susceptible to ***Anogeissus leiocarpus, Punicar granatum*** (Bark) and ***Biota orientalis***.

This study revealed the need for more studies on MRSA. Further studies are olso needed to explore the activity of benzinic and chloroformic extract of all test medicinal plants. The methanolic extract, especially those showing high antibacterial efficacy sould be subjected to rigorous testing to determine their usefulness as alternative antibacterial agents.

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Chapter One

Introduction and Literature Review

1. Introduction and Literature Review

1.1. Medicinal Plants: Past and Future

Man has been using medicinal Plants as source of treatment for his ailments over the millennia. Therfore, a wealth of ethno-medical knowledge that was passed down from generation to generation through apprenticeship and clerkship has been made available today and awaiting extensive exploration. Moreover, this ethnopharmacological knowledge would reduce empiricism and increase the chance of success in new drug-finding efforts (Patwardhan, 2005; Cordell and Colvard, 2005). This can only be attained only if academia and research institutions had the full support of governments and regional and international organisations. Such support is vitally important due the ever diminishing interest of the pharmaceutical industry in natural products which is believed to be chemically complex and lacking assurance of renewable supplies.

However, the limited studies that have been done so far, have already lead to discovery of various new active compounds (Runyoro 2006) of known therapeutic properties (Harborne and Baxter, 1995). The results of such studies indicated that the medicinal plants that our ancestors have used, could be an important source of new biologically active compounds that can be used for treating various diseases (Clardy and Walsh, 2004). Antimicrobial properties of various medicinal plants that can be used for treating infectous deseases have been reported (Nimri et al., 1999; Saxena and Sharma, 1999, Srinivasan, 2001).

Even today medicinal plants are still being used to treat various diseases worldwide, especially in developing countries including Sudan, and in

areas of limited access to modern medicines in particular, (Iwu et al., 1999,). However, in the industrialised countries where full access to modern medicines is available to everybody, herbal remedies still continue to play a role in the cure of diseases (Tabuti et al., 2003). It is estimated that up to a quarter of all prescriptions contains one or more components of plants origin (Farnsworth1990), and about 50% of the modern drugs either contain plant materials or these materials have provided the model for them (Harbone, 1998).

1.2. Mechanisms of control of diseases and wound by medicinal plants extracts

Reactive oxygen species are involved in a number of degenerative diseases. It was found that delaying or preventing the onset of these diseases (arthrosclerosis, cancer, cirrhosis and diabetes) and in healing wounds (Aboutwerat et al., 2003) was attributed to plant antioxidants constituents such as tannins, lignans, stableness, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins. These diseases are caused by reactive oxygen species; therefore the redox properties of the antioxidant constituents allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OHÅ) or super-oxide radical (OÅ2) scavengers (Robards et al., 1999; Govindarajan et al. 2005). In addition to that, they were found to act as strong chelators of metal ions (Rice-Evans et al. 1995). Therefore, it was concluded that consumption of antioxidants rich fruits and vegetables would control these diseases (Sun et al. 2002).

1.3. Wounds and Abscesses

1.3.1. Wounds

Wounds are a type of injury in which the skin is torn, cut or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin.

The skin is home to a wide variety of bacteria that causes the host no problems when the skin is intact. However, when disrupted with a wound, the skin provides ideal conditions for bacteria to become pathogenic, multiply and 'invade' through that weak point. Wounds provide an ideal culture medium for the growth of bacteria because they have the optimum temperature for bacterial growth; a frequent supply of nutrients in the form of organic debris; and a moist environment. The presence of bacteria in a wound does not necessarily indicate that infection has occurred or leads to impairment of wound healing. However, increased bacterial load can lead to reduced or no healing (Dealey 1995; Collier 2001).

Wounds being acute, post-operative or chronic such as pressure ulcers, leg ulcers and acute wounds that have been present for more than six weeks (Dealey 1995), can be classified depending on the amount of bacteria present and the type of pathogens likely to be present. The main types are: contaminated wounds (Bacteria are present in low numbers but there is no multiplication); colonised wounds (There is multiplication of bacteria but no host reaction.); infected wounds (There is deposition and multiplication of bacteria in the tissue with an associated host reaction). It has been considered that a bacterial content of greater than

105//g tissue comprises clinical infection and may delay healing (Fowler 1990).

The indiscriminate use of topical antibiotics for the routine treatment of colonised or infected wounds (Drug and Therapeutics Bulletin 1991), can provoke delayed hypersensitivity reactions super infections and select for resistance (Kaye 2000) which is a serious problem.

1.3.2. Absesses

An abscess is a collection of pus (dead neutrophils) that has accumulated in a cavity formed by the tissue as a defensive reaction to prevent the spread of infectious materials caused by organisms (e.g. bacteria or parasites) or other foreign materials (e.g. splinters or bullet wounds) to other parts of the body through the abscess wall, or capsule, that is formed by the adjacent healthy cells. However, such encapsulation tends to prevent immune cells from attacking bacteria in the pus, or from reaching the causative organism or foreign object. Therefore, abscesses rarely heal themselves, they require a prompt medical attention at the first suspicion of an abscess, and especially that it could be fatal when it compresses vital structures such as the trachea. Abscesses do not generally need to be treated with antibiotics, but they will require surgical intervention, debridement and curettage (Leaper 2007). After draining (e.g. lancing) abscesse, painkillers and possibly antibiotics may be required (Green et al. 2000). Antibiotic therapy alone without surgical drainage of the abscess is seldom effective due to antibiotics often being unable to get into the abscess and their ineffectiveness at low pH levels.

As *Staphylococcus aureus* is a common cause, an anti-staphylococcus antibiotic such as flucloxacillin or dicloxacillin is used. With the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* MRSA, these traditional antibiotics may be ineffective; alternative antibiotics effective against community-acquired MRSA often include clindamycin, trimethoprim-sulfamethoxazole, and doxycycline.

Recurrent abscesses are often caused by community-acquired MRSA, which is resistant to most beta lactam antibiotics, but unlike hospital-acquired MRSA that may only be sensitive to vancomycin IV, they may be sensitive to alternative antibiotics, e.g., clindamycin (Cleocin), trimethoprim-sulfamethoxazole (Bactrim), and doxycycline (Abraham et al. 1997).

However, recently, efforts to find antibacterial agents from medicinal plants are gathering pace. Indeed, this study is part of such efforts, where, the antibacterial properties of twelve medicinal plants have been investigated.

1.4. Methods of Medicinal Plants Investigation

To study medicinal plants, various methods have been reported:

1. Phytochemical approach that searches for classes of secondary metabolites containing various antimicrobial substances such as alkaloids, isothiocyanates, etc. This approach is considered easy to perform, hence its popularity.
2. Collection of all available plant parts, irrespective of prior knowledge and experience. This approach is considered expensive and laborious.

3. Selection of plants based on published reports on their antimicrobial activities.
4. Ethnomedical approach, where selection of plants and focused evaluation is based on oral or written information on the medicinal use of a plant, which can be obtained from organised traditional medical systems (Ayurveda, Unani, Kampo, shamanism and traditional Chinese medicine), herbalism and folklore.
5. Non-systematic ethnomedical approach (serendipity), where plant selection is based on ethnomedical use, but where the revealed bioactivity is unexpected and depends on chance (Fabricant and Farnsworth, 2001).

Ethno-directed method, which is widely used by researchers, was chosen in this study for the fundamental role it would play in biodiversity prospecting in general (Cordell 2000) and in this part of the world in particular. The main objectives were to add-on to the inventory of the medicinal species that are traditionally used in the Sudan, and evaluate the twelve plant species' antibacterial activities.

1.5. Study objectives

1.5.1. General Objectives:

Extraction and test of twelve medicinal plants, and characterisation of their bioactivity, to add-on to the inventory of the medicinal plant species that are used traditionally in treating infectious diseases.

1.5.2. Specific Objectives:

1. Carrying primary screening assays using standard bacterial agents to a selection of extracts with promising

pharmacological action, the so-called “hits”, that are worthy of following-up.

2. Isolation of bacterial organisms prevalent in wounds and abscesses, and determination of their susceptibility to the extracts of the test medicinal plants, adopting specialised in vitro bioassays to define “lead” status.

1.6. Test Medicinal Plants

1.6.1. Piper cubeba L.

1.6.1.1. Habitat, Origin and Botanical Description

Piper cubeba L. is of the Order Piperales and the Family Piperaceae. It is known in English as Jawa Peppercorn and Jawanese Pepper, and known in Arabic as “Kabaaba” (كَبَابَة) and "Habb-ul-Uruus" (حب العروس). It inhabits the Isles of the Indian Ocean and is cultivated in various parts of the world. The berries of *Piper cubeba L.* are commonly known as cubeb and are important as a source of pepper (the dried berries) for the worldwide spice market (Usia et al., 2005 b). The fruit is pungent and bitter with a strong terpene aroma.

1.6.1.2. Major Chemical Constituents

Phytochemical and biological investigations have shown three groups of secondary metabolites from the berries of *Piper cubeba L.*, i.e. alkaloids, lignans composed of cubebin and several related compounds: hinokinin, clusin, dihydroclusin, dihydrocubebin and more, and terpenoids (essential oil) composed of monoterpenes (sabinene 50%, carene, α-thujene, 1,4-cineol and 1,8-cineol) and sesquiterpenes (copaene, α- and β-cubebene, δ-cadinene, caryophyllene, germacrene, cubebol). The

lignans and the essential oil have been more intensively investigated, since *Piper cubeba L.* accumulates both groups of compounds in relatively high amounts. On the other hand, reports showed that, piperine is an abundant alkaloid in the berries of this species (Parmar et al., 1997).

1.6.1.3. Medicinal and Pharmacological Activities

a. Antibacterial activity

The crude ethanol extract from Piper cubeba L. seeds, cubebin and its semi-synthetic derivatives, (-)-hinokinin were found to be effective against oral pathogens, namely against Streptococcus salivarius, Streptococcus mitis and Enterococcus faecalis (Silva et al. 2007).

b. Other Activities

***Piper cubeba L.* is used in traditional medicine to treat gonorrhea, dysentery, syphilis, abdominal pain, diarrhea, enteritis and asthma (, 2001).**

Recent studies have shown that Methanol and water extracts of *Piper cubeba L.* berries posses an inhibitory effect against the hepatitis C virus (Hussein et al., 2000), and anti-inflammatory, antioxidant, antiallergic and analgesic activities (Choi and Hwang, 2003, 2005).

Lignans are an important group of secondary metabolites of *Piper cubeba L.* in terms of their biological activity. More than twenty lignans have been reported (Parmar et al., 1997; Usia et al., 2005a), to have biological activities. Some were found to be effective against enzymes such as cytochrome P450 that were responsible for metabolism of currently used drugs (Usia et al., 2005 a,b). Other Lignans were found to

have antifeedant, anti-inflammatory, analgesic and trypanocidal properties.

Yatein, hinokinin, cubebin, dihydrocubebin were found to have antifeedant activity against a number of stored product insects (Harmatha and Nawrot, 2002). Cubebin has been shown to posses anti-inflammatory, analgesic and trypanocidal activities (Borsato et al., 2000; Bastos et al., 2001; De Souza et al., 2005).

Some of the above mentioned lignans could be used as biosynthetic precursors. Hinokinin because of its structural relationship, it can be synthesised using cubebin as precursor (Da Silva et al., 2005). Yatein could be used as a biosynthetic precursor of deoxypodophyllotoxin and podophyllotoxin that are well known for their anticancer properties.

1.6.2. Acorus calamus L.

1.6.2.1. Habitat, Origin and Botanical Description

Acorus calamus L. of the order (*Arales*) and the family *Araceae*, known in English as Sweet Flag, Myrtle Flag and Sweet Sedge, and known in Arabic as "Athareera" (الذريره). It is a semi-aquatic plant growing in damp, marshy places throughout the world. It is a perennial, aromatic herb with creeping rhizomes. The leaves are long, slender, sword-shaped and simple, arising alternately from the horizontal rhizomes. These are longitudinally fissured with nodes, somewhat vertically compressed and spongy internally. Flowers small, fragrant, pale green in a spadix; fruits are a three-celled fleshy capsule.

1.6.2.2. Major Chemical Constituents

Known phytoconstituents of *Acorus calamus L.* parts used: include glucoside acorin, alkaloid, Emetic, 1.5–3.5% essential oil and methyl isoeugenol (Harborne and Baxter, 1995)

Essential oils found in *Acorus calamus L.* include β-Asarone (isoasarone) which is usually the major constituent but is present in highly variable proportions and occasionally absent (Sinha 2001); a-Asarone, elemicine, cis-isoelemicine, cis and trans isoeugenol and their methyl ethers; camphene; P-cymene; β –gurjunene; a-selinene; β –cadinene; camphor; terpinen-4-01; a-terpineol and a-calacorene; acorone; acorenone; acoragermacrone; 2-deca-4,7-dieno1; shyobunones; isoshyobunones; calamusenone; linalool and pre-isocalamendiol are also present.

Other chemicals found in *Acorus calamus L.* include acoradin, galangin, 2,4,5-trimethoxy benzaldehyde, 2,5-dimethoxybenzoquinone, calamendiol, spathulenol and sitosterol.

1.6.2.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

Growth of cultured Gram-positive and Gram-negative organisms was found to be inhibited significantly by an extract of the *Acorus calamus L.* rhizome. Also, consistent and gradual decrease in replication of standard cultures of *Staphylococcus aureus* , *Escherichia coli* and *Shigella flexneri* was observed after treatment with *Acorus calamus L* essential oil (Singh .al. et, 2001, Mc Gaw et. al., 2002, Parekh, and Chanda 2007).

b. Other Activities

Acorus calamus L. has long been known for its medicinal value. It originated in Europe but has been extensively used in Ayurveda, particularly to enhance memory. Its powder mixed with ghee (coocked clear cream) is given ritually in India to newborn babies on the seventh day to improve the intellect and speech development. In China it is used in a similar way, to improve speech and aid recovery from stroke. The powder of Acorus calamus L is sometimes blown into the nose of a patient in a coma to help regain consciousness. There are several polyploid varieties to be found, some of which do not contain the toxic constituent β-asarone, and these are preferable for medicinal use.

Acorus calamus L. **rhizomes are used in many different disorders, mainly as a nerve stimulant, to enhance memory and as an aromatic digestive. It is considered to be the remogenic, rejuvenative and sedative. Other uses for the plant include diuretic, expectorant, decongestant, anti-inflammatory, aphrodisiac, anticonvulsant and antibacterial. It has also been used in the treatment of epilepsy, chronic diarrhoea, dysentery, bronchial catarrh, intermittent fever and certain tumours (Zanoli et. al.1998). The main activities of *Acorus calamus L.* are summarised as:**

1. **Antiulcer and cytoprotective activity: The ethanolic extract of the rhizome has shown to protect the gastroduodenal mucosa against injuries caused by indomethacin, reserpine and cysteamine, and also in a pyloric ligation model.**
2. **Antispasmodic activity: Experiments on the ileum, uterus, bronchial muscles, tracheal chain and blood vasculature showed the relaxant and antispasmodic activity of β -asarone and the essential oil of the**

rhizome. The rhizome is useful in the treatment of diarrhoea and dysentery, combined with ginger for relief in flatulent colic.

3. Analgesic activity: The essential oil and alcoholic extract of the rhizome were shown to possess analgesic properties and also mild hypotensive and sedative action.
4. Anti-inflammatory activity: An extract of the rhizome was studied in acute, chronic and immunological models of inflammation, including carrageenan-induced rat paw oedema, and compared with the activity of hydrocortisone. The extract showed significant antiinflammatory activity with a reduction of up to 44%. The essential oil is also an effective antiinflammatory agent and a coconut oil extract of the rhizome produced a 45% inhibition of carrageenan-induced rat paw oedema and 61 % inhibition using the granuloma pouch method.
5. Anticonvulsant activity: A polyherbal compound containing rhizome of Acorus calamus L. as one of the ingredients has been reported clinically to reduce epileptic attacks in patients by up to 50%. Treatment continued for 6 months resulted in cure in 66 out of 88 patients and no repeat episodes were reported after 2 years of the treatment. The isolated constituents of the rhizomes, asarone and β-asarone, showed anticonvulsant activity in experimental models. There was a decrease in sociability scores with a reduction in anticholinergic action. Experiment on animal models also revealed the depressant action of the essential oils and the crude alcoholic and aqueous extracts of the rhizomes.

1.6.3. *Anogeissus leiocarpus*

1.6.3.1. Habitat, Origin and Botanical Description

Anogeissus leiocarpus is of the order Myrtales and the family Combretaceae. It is known in English as Axlewood tree and in Arabic as "Al-Sahab" (الصحب), it is a graceful tree of the Sahel to forest zones, straight tapering boles up to 20 m high, with greyish white and scaly bark, it branches from low down often gregarious and effectively weeding out competing grasses (Dalziel, 1937). Its leaves are alternate; ovate-lanceolate; apex acute; base-auenuate; pubescent beneath. Inflorescences are globose heads, yellow and petals are absent. Fruits are globose, cone-like heads, broadly winged, coriaceous, and dark grey.

1.6.3.2. Major Chemical Constituents

The *Anogeissus leiocarpus* parts were generally found to contain important bioactive substances such as glycosides, phenols, tannins, saponins, alkaloids, steroids, ellagic acids and anthraquinones. These agents may be responsible for the antibacterial activity of this plant (Mann et. al. 2008).

1.6.3.3. Medicinal and Pharmacological Activities

a. **Antibacterial Activity**

The extract of *Anogeissus leiocarpus* demonstrated appreciable activity against aerobic *Staphylococci* and *Streptococci*, and against the facultative Gram negative rods (*Escherichia*, *Citrobacter*, and *Enteroabacter spp.*). Although, gram-negative organisms tend to have a higher intrinsic resistance to most antimicrobial agents, *Anogeissus*

leiocarpus is impressively active against the Gram-negative organisms (Taiwo et. al.1999, Ndukwo et. al. 2005). This confirms that *Anogeissus leiocarpus* and many other plants used in Africa and elsewhere as chewing sticks can be regarded as candidates for extraction of substances, which may be used as antibacterial agents for treatment of oral infections caused by a broad spectrum of pathogenic organisms. However, comprehensive toxicological investigations must be carried out beforehand as toxicity of *Anogeissus leiocarpus* to mice has been reported (Rotimi et. al. 1988).

In vitro investigations showed that the extracts of combination of the three parts of Anogeissus leiocarpus have antibacterial activity against clinical isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The highest antibacterial activity was observed against Staphylococcus aureus (Mann et. al. 2008).

a. Other Activities

Anogeissus leiocarpus leaves are used as fodder for livestock. It is also used in traditional medicine as a remedy for many ailments of both livestock and man, which include helminthosis, schistosomiasis, leprosy, diarrhoea and psoriasis (Onyeyili, 2000). Roots are commonly used as chewing sticks in oral hygiene in Africa including the Sudan and other parts of the world; and the decoction of the barks are used against cough.

1.6.4. Calendula arvensis

1.6.4.1. Habitat, Origin and Botanical Description

Calendula arvensis is of the order *Compositae* and the family *Asterales*. There are two known species, *Calendula officinalis* and *Calendula arvensis*, and known in English as Field marigold, Garden marigold, Goldbloom, Holligold, Maravilla and Marybud; and in Arabic is known as "Alhanwa" (الحنوه). It is an annual herbaceous self-seeding plant bright yellow or gold flowers that bloom from May until October; it grows to about two feet tall with multiple branches. Its flowers follow the sun; hence, it was linked to the astrological sign of summer, Leo. *Calendula arvensis* is native to central Europe and the Mediterranean; it grows readily in sunny locations throughout North America Europe. It also, grows in Asia and Africa including the Sudan, and it was cultivated by the Egyptians, Greeks, Hindus and Arabs, and has been used in traditional medicine since the 12th century.

1.6.4.2. Major Chemical Constituents

Six different saponin glycosides are identified in *Calendula arvensis* (Vidal-Ollivier and Balansard, 1989). These compounds are derivatives of oleanolic acid and are known as glycosides A-D, D2, and F. Several glucuronides are also identified in these species (Szakiel et. al, 1990). The content of oleanolic acid and its glycoside and glucuronide derivatives are found in the flowers and leaves (Vidal-Ollivier and Balansard, 1989). The flowers also contain carotenoids, mainly citroxanthine, flavochrome, beta-carotene, and flavoxanthine, that produce a golden yellow color (Anon, 1998), as well as isorhamnetin and quercetin (Bezakova et al.1996). Three different faradiol esters were also found in the flowers (Zitterl-Eglseer et al. 1997). In *Calendula*

arvensis, sesquiterpene glycosides (De Tommasi N and Pizza 1990, Ahmed et al. 1993) and triterpenoid saponins (De Tommasi 1991) were isolated from the aerial parts of the plant. Among the sesquiterpene glycosides are the epicubebol and alloaromadentol compounds, which contain a cyclopropane ring structure (De Tommasi N and Pizza 1990, Ahmed et a., 1993). Oil that contains volatile components along with carotenoids can also be extracted from the blossoms and the small dry fruits (Baranyk 1995) of *Calendula arvensis*.

1.6.4.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

Calendula arvensis as antibacterial, *in vitro* studies gave conflicting results. Some studies showed antibacterial effects against *Bacillus subtilits*, *Escherichia coli*, and *Staphylococcus aureus* (Janssen et al, 1986); the arvensosides B and D were somewhat active against *Trypanosoma brucei* (Dvorzak 1989). In other studies, *Calendula arvensis* was inactive against *Aerobacter aerogenes*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus morganii, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Strep faecalis, Staphylococcus aureus* (Acevedo et al. 1993).

b. Other Activities

Calendula arvensis is used in traditional medicine (Vidal-Ollivier and Balansard 1989) as an anti-inflammatory agent against sunburn, for example, and as an antipyretic (Vidal-Ollivier and Balansard 1989, De Tommasi et al., 1990). It is taken internally to assist and promote menstrual discharge in women, in promoting wound healing (Vidal-Ollivier and Balansard, 1989, Anon, 1998, Kathi 1999), and in cancer

treatment (De Tommasi et al., 1990, Kathi and Kember 1999). *Calendula arvensis* is known to accelerate the formation of new tissue, therefore the flowers were made into extracts, tinctures, balms and salves and applied directly to the skin to treat various skin conditions, such as dry skin in babies, eczemas, and cuts (Anon, 1998, Kathi and Kember 1999).

In Italian folk medicine *Calendula arvensis* is used as an antipyretic and anti-inflammatory. Teas made from it are used as eye washes, gargles or compresses to treat conjunctivitis, pharyngitis, aphthous stomatitis and gingivostomatitis, diaper rashes and other inflammatory conditions of the skin and mucus membranes (Fleming 1998). In India, herbal compounds including *Calendula arvensis* are used topically to treat hemorrhoids. *Calendula arvensis* cream alone or in combination with other remedies is also a favorite homeopathic remedy to treat abrasions and minor burns. Dried *Calendula arvensis* petals are used in the spice trade as an inexpensive alternative to saffron and are used in many ointments to enhance their appearance by adding a gold colour. Like other members of the daisy family, the dried flowers have also been used as an insect repellent. Some herbalists combine *Calendula arvensis*, comfrey, and echinacea and St. John's wort in a cream or ointment as an all-purpose skin salve (Kember 1999).

***Calendula arvensis* has Gastrointestinal/hepatic activity, namely against Chronic colitis and ulcers (Chakurski et al 1981). Also, it has reproductive activity, it has estrogenic and uterotonic effects. In vitro studies showed that its extracts exhibited moderate uterotonic effects in isolated rabbit and guinea pig uterine horn tissues (Shipochliev 1981). *Calendula arvensis* has immune modulation activity; it is a immunostimulant and anti-inflammatory. As an Immunostimulant, in vitro**

studies showed that its polysaccharides might stimulate phagocytosis (Wagner et al, 1985). As an anti-inflammatory, *in vitro* studies showed that *Calendula arvensis*' glycosides inhibited lipoxygenase activity (Bezakova et al. 1996). In several studies, *Calendula arvensis'* triterpenoids (especially the faradiol monoester) reduced experimentally induced inflammation in mice (Yasukawa et al. 1993, Loggia et al. 1994, Akihisa et al. 1996, Zitterl-Eglseer et al. 1997). Rats with long-standing ocular inflammation improved when treated with *Calendula arvensis* eyewashes; however, there was no comparison group in this study. Anecdotal cases report decreased pain and inflammation in postmastectomy patients (Smith 1983) and in children with chronic suppurative otitis media.No controlled trials have been reported.

Calendula arvensis has antimicrobial (antiviral, antibacterial, antifungal) activity. As antiviral, studies gave conflicting results. Its *sesquiterpene glycosides* inhibited replication of rhinovirus and Herpes virus (De Tommasi et al, 1990, 1991); *Calendula arvensis* extracts also displayed some anti-HIV activity, including a dose-response effect against reverse transcriptase activity (Kalvatchev 1997); another study demonstrated activity against Herpes simplex and influenza viruses. However, other studies showed no antiviral activity against polio, vaccinia, influenza or Herpes viruses.

1.6.5. *Nauclea latifolia*

1.6.5.1. Habitat, Origin and Botanical Description

Nauclea latifolia is of the order *Rubiales* and the *Rubiaceae* family, known in English as Pin cushion tree, and in Arabic as the "Karmadoda" (كرمدودة). It is glabrous shrub or small tree with drooping branches. Leaves are opposite, elliptic-oblong, apex acuminate, base cuneate to

subcordate, margin entire and Inflorescences terminal globose heads. Its fruits drupes, globose with granulated surface. *Nauclea latifolia* is a widely distributed savanna plant. It is found in the forest and fringe tropical forest.

1.6.5.2. Major Chemical Constituents

Key constituents of *Nauclea latifolia* are indole-quinolizidine alkaloids and glycoalkaloids, tannins and sapponins (Iwu 1993, Iwu et al 1999, El-Mahmood et al. 2008).

1.6.5.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

Studies showed that *Nauclea latifolia* has antibacterial activity against gram positive and negative bacteria and antifungal activity (Iwu 1993, Iwu et al 1999). It is most effective against *Corynebacterium diphtheriae*, *Staphylococcus aureus Streptobacillis* sp., *Streptococcus sp.*, *Neisseria sp.*, *Pseudomonas aeruginosa*, *Salmonella spp* and *Escherichia coli* (Deeni 1991). *Escherichia coli*, though a Gram-negative bacterium, has been reported to be sensitive to extracts of *Nauclea latifolia* (Omer et al., 1998, Abreu et al., 1999, Umeh et al 2005).

b. Other Activities

Medicinal uses of *Nauclea latifolia* are tonic and fever medicine, chewing stick, toothaches, dental caries, septic mouth and malaria, diarrhea and dysentery.The maceration of the barks is used to irradicate tape worms (Lamidi 1995, Iwu et al 1999).

1.6.6. *Petasites hybridus*

1.6.6.1. Habitat, Botanical Description Origin and Uses

Petasites hybridus **of the Order Asterales and the Family *Asteraceae*. It is known in Enlglish as Butterbur which is attributed to the large leaves being used to wrap butter during warm weather (Butterbur 1971). Other names include Bog rhubarb, Devil's hat and Pestilence. In Arabic it is known as "Fikhet" (فكهت). It is herbaceous perennial plant usually found in wet, marshy ground, in damp forests, and adjacent to rivers or streams. It is native to Europe and Northern Asia, but found throughout Europe as well as parts of Asia and North America. The flowers are produced in the early spring, before the leaves appear; flowering shoots up to 40 cm tall and the flowers are pale pink, with several inflorescences clustered on a 5-20 cm stem. The leaves are large, on stout 80-120 cm tall stems, round, with a diameter of 40-70 cm (USDA, NRCS 2006, Monograph, 2001).**

1.6.6.2. Major Chemical Constituents

The main active constituents that are isloted from the Petasites hybridus rhizomes, roots, and leaves are two sesquiterpenes, petasin and isopetasin. Petasin is responsible for the antispasmodic properties of the plant by reducing spasms in smooth muscle and vascular walls, in addition to providing an anti-inflammatory effect by inhibiting leukotriene synthesis. Prostaglandins are important mediators in the inflammatory process and isopetasin's positive impact on prostaglandin metabolism contributes to the effectiveness of Petasites hybridus extracts. Extracts of the plant also contain volatile oils, flavonoids, tannins, and pyrrolizidine alkaloids. As these alkaloids are believed to be toxic to the

liver and carcinogenic in animals, extracts in which contain the pyrrolizidine alkaloids must be removed (Seeram et al. 2005).

1.6.6.3. Medicinal and Pharmacological Activities

Extract from ***Petasites hybridus*** with three main compounds oxopetasan esters, petasin and isopetasin were found to inhibit the biosyntesis of the vasoconstrictive peptide leukotrienes, hence contributing to gastroprotection and spasmolyticactivity (Bickel et al. 1994).

Traditional medical uses of ***Petasites hybridus*** include its applications as a diuretic and muscle relaxant, and in treating coughs, fever, wounds, stammering, headaches, asthma and stress (USDA, NRCS 2006, Monograph, 2001). Currently, its main uses include prophylactic treatment of migraines, and as an antispasmodic agent for chronic cough or asthma. It has also been used successfully in preventing gastric ulcers, and in treating patients with irritable bladder and urinary tract spasms (Mauskop 2000).

1.6.7. Salvia officinalis

1.6.7.1. Habitat, Origin and Botanical Description

Salvia officinalis is of the order *Lamiales* and the *Labiatae* family, it is known in English as the Garden sage, Kitchen sage, and Dalmatian sage, and in Arabic is known as "Mariamia" (مريمية). It is a small evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is in leaf all year, in flower from June to August, and the seeds ripen from August to September. The flowers are hermaphrodite. It is native to the Mediterranean region. It grows on dry banks and stony places (Polunin 1969), usually in limestone areas and often where there is a very little soil (Grieve 1984).

Salvia officinalis is much cultivated as a kitchen and medicinal herb. Common sage is also grown in parts of Europe, especially the Balkans for distillation of the essential oil.

1.6.7.2. Major Chemical Constituents

Salvia officinalis contains essential oils including cineole, borneol, thujone, α-thujene, α-pinene, camphene, 2-β-pinene, β-myrcene, α-terpinene,1,8-cineole, γ-terpinene, α-thujone, β-thujone, camphor. 1-borneol, 1,4 terpineol, endobornyl acetate, caryophyllene, β-selinene, veridiflorol and manool (Miladinović and Miladinović 2000). Its leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavone glycosides, and estrogenic substances.

1.6.7.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

The crude extracts of *Salvia officinalis* posses (Velickovic et al 2003) antibacterial activities towards the Gram-positive bacteria (*Bacillus subtilis* and MRSA) but no activity was shown against the Gram-negative bacteria (enterohemorrhagic *Escherichia coli* and *Pseudomonas aeruginosa*) which was attributed to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan 1998, Abu-Shanab 2004).

In another study the essential oil from leaves of *Salvia officinalis* showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* , *Escerichia coli* and *Salmonella enteritidis* (Velickovic et al 2003) , as well as *Klebsiella pneumuniae*, *Proteus mirabilis*, *Morganella morganii*,

Enterobacter aerogenes, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* (Miladinović and Miladinović,2000).

b. Other Activities

Salvia officinalis has been recommended at one time or another for virtually every ailment. Modern evidence supports its effects as an antihydrotic, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic, and tonic. In a double blind, randomized and placebo-controlled trial, sage was found to be effective in the management of mild to moderate Alzheimer's disease (Akhondzadeh et al. 2003).

Salvia officinalis used internally for indigestion, gas, liver complaints, excessive lactation, excessive perspiration, excessive salivation, anxiety, depression, female sterility, menopausal problems; and externally for insect bites, throat, mouth, gum, skin infections, vaginal discharge.

The crude extracts of *Salvia officinalis* showed antifugal activity against *Aspergillus niger* (Velickovic et al 2003). The leaves of *Salvia officinalis L*. are reported to have a wide range of biological activities, such as anti-bacterial, fungistatic, virustatic, astringent, eupeptic and anti-hydrotic effects. Also it was found to potentiate memory retention and it has an interaction with muscarinic and nicotinic cholinergic systems that is involved in the memory retention process (Eidi et al).

1.6.8. *Punica granatum L.*

1.6.8.1. Habitat, Origin and Botanical Description

Punica granatum L. **is of the order *Myrtales* and the *Punicaceae* family, it is known in English as Pomegranate, and in Arabic as "Alroman" (الرمان). It is a neat, rounded shrub or small tree that can grow to 20 or 30 ft., but more typically to 12 to 16 ft. in height. Dwarf varieties are also known. It is usually deciduous, but in certain areas the leaves will persist on the tree.**

Punica granatum L. **leaves are glossy leathery and narrow and lance-shaped. The trunk is covered by a red-brown bark which later becomes grey. The branches are stiff, angular and often spiny, and they have the tendency to sucker from the base. The flowers are attractive scarlet, white or variegated and are over an inch across and have 5 to 8 crumpled petals and a red, fleshy, tubular calyx which persists on the fruit. The flowers may be solitary or grouped in twos and threes at the ends of the branches. *Punica granatum L* is self-pollinated as well as cross-pollinated by insects. The fruit is nearly round, 2-1/2 to 5 in. wide fruit is crowned at the base by the prominent calyx. The tough, leathery skin or rind is typically yellow overlaid with light or deep pink or rich red. The interior is separated by membranous walls and white, spongy, bitter tissue into compartments packed with sacs filled with sweetly acid, juicy, red, pink or whitish pulp or aril. High temperatures are essential during the fruiting period to get the best flavour (Morton 1987; Stephen 1990).**

Punica granatum L. **prefer a semi-arid mild-temperate to subtropical climate and are naturally adapted to regions with cool winters and hot summers. A humid climate adversely affects the formation of fruit. The tree can be severely injured by temperatures below 12° F. It is native of**

the area spaning from Iran to the Himalayas in northern India and was cultivated and naturalised over the whole Mediterranean region since ancient times. It is widely cultivated throughout India and the drier parts of Southeast Asia, Malaya, the East Indies, tropical Africa and America (Butterfield 1963).

1.6.8.2. Major Chemical Constituents

Punica granatum L. major class of phytochemical includes the polyphenols, flavonoids, condensed tannins and hydrolysable tannins. Hydrolysable tannins are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity (Gil et al. 2000). When crushed and dried, the seeds produce oil with 80% punicic acid, the 18-carbon fatty acid, along with the isoflavone genistein, the phytoestrogen coumestrol, and the sex steroid estrone. The seed coat of the fruit contains delphinidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3,5-diglucoside, pelargonidin-3-glucoside, and pelargonidin-3,5-diglucoside with delphinidin-3,5-diglucoside being the major anthocyanin in *Punica granatum L* juice (Afaq et al. 2004). *Punica granatum L* extract has also been shown to protect from NSAID and ethanol-induced gastric ulceration (Ajaikumar et al. 2005).

1.6.8.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

Punica granatum L. juice has shown to inhibit serum angiotensin-converting enzyme, and hence reducing systolic blood pressure (Aviram and Dornfeld 2001). Also, it has shown to inhibit viral infections (Neurath et al. 2004), and may have antibacterial effects against dental plaque

(Menezes et al. 2006). In the Midle East, *Punica granatum L*. are used to cure mouth ulcers, where the syrup is sieved boiled to form molasses and then applied directly to the mouth ulcer.

b. ***Other Activities***

Punica granatum L. is used in traditional medicine for the treatment and prevention of arthritis and other inflammatory diseases. Rececent studies showed that standardised extracts of *Punica granatum L*. fruit possess anti-inflammatory and cartilage sparing effects (Ahmed et al. 2005), inhibits the proliferation of human cancer (Seeram et al. 2005; Afaq et al. 2004; Aviramet al. 2000; Syed et al. 2006; Adams et al. 2006) and it reduces the growth of prostate tumors and the levels of prostate-specific antigen (Malik 2005). Also, it was reported that consumption of *Punica granatum L*. may have cholesterol lowering and cardiovascular and other chronic diseases preventing effects (Esmaillzadeh et al.2006; Rosenblat , et al. 2006; de Nigris et al. 2007; Afaq et al. 2005).

1.6.9. Cissus petiolata

1.6.9.1. Habitat, Origin and Botanical Description

Cissus petiolata of the order *Vitales* and the *Vitaceae* family, it is known in English as (Bitter apple and Bitter gourd), and in Arabic as "Irg-alhagar" (عرق الحجر). *Cissus petiolata* grows in fringing forest and spreads worldwide, but mainly in tropical and subtropical regions. In Africa, it spreads from Guinea Republic to Ethiopia and southwards through the Congo, Uganda and Tanzania and reaching its southern limit in Angola and Mozambique. It is a vigorous climber; stems quadrangular, glabrous, becoming woody when old and developing longitudinal corky wings up to 1·2 cm. broad on the angles; tendrils simple. Leaves are

simple, glabrous or with a few hairs on the petioles and lower surface of the leaf when young; petiole up to 10·8 cm. long but usually shorter; leaf-lamina up to 13 × 13 cm. but usually somewhat smaller, circular to broadly ovate. Flower-buds are conical, up to 3 mm. long. Calyx 1 mm. long, pubescent when young. Petals are glabrous, ovary glabrous; style 1–2 mm long. Fruit is 8 mm in diameter, glabrous. Seeds are up to 4 per fruit, only 1 usually becoming fully developed, smooth, 6 mm long with one well-marked crest and 2 subsidiary ones.

1.6.9.2. Medicinal and Pharmacological Activities

Cissus petiolata is used as medicine for menstrual cycle; tumours, cancers (Bark), and stomach troubles (Root) and root-sap is used for ear treatment. It is used traditionally for fungal infections treatment (Omar et al 2006). Also, Cissus petiolata extracts were found to have toxic activity against cercariae and miracidia of Schistosoma mansoni (Elsheikh et al 1990)

1.6.10. Moringa peregrine

1.6.10.1. Habitat, Origin and Botanical Description

Moringa peregrine is of the Order Brassicales and the Family Moringaceae. It known in English as the wild Drum-stick tree and in Arabic is known as "Yusor, Al Yassar, and Al Ban" (اليسر، البان) "shagarat al rauwãq" (شجرة الراواق). Moringa peregrine occurs naturally in arid or semi-arid region, it spreads from the Dead Sea area and along the Red Sea coasts to northern Somalia and around the Arabian Peninsula to the mouth of the Persian Gulf. It grows on rocky slopes of wadis and gullies, up to 850 m altitude, sometimes on nearly bare rock with a strongly reduced root system.

Moringa peregrine is a shrub or small tree up to 10 m tall, with tuberous rootstock; bole up to 40 cm in diameter; bark grey, purple-grey or bright brown; crown ovoid; branches terete, slender, young stems grey-white or waxy blue-green; twigs brittle. Leaves alternate, in bunches at the ends of branches, 15–40 cm long, 2–pinnate, with 2–5 pairs of pinnae; leaflets opposite or alternate, obovate, oblanceolate or spatulate, 3–20(–35) mm × 2–10(–13) mm, base cuneate to rounded, apex rounded or notched, grey or waxy green. Flowers bisexual, slightly zygomorphic, 5-merous, white with purple heart or pink-flushed, sometimes scented; pedicel 2–9 mm long, jointed; sepals free, oblong to lanceolate, 7–9 mm × 1.5–3 mm, acuminate, hairy on both surfaces; petals free, narrowly oblong, obovate or spatulate, 8–15 mm × 2–5 mm, hairy inside; stamens 5, free, 4.5–7 mm long, alternating with 5 staminodes, 4–5 mm long; ovary superior, shortly stalked, cylindrical, hairy, 1-celled, style slender. Fruit an elongate capsule (10–) 32–39 cm × (1–)1.5–1.7 cm, somewhat trigonous, slightly narrowed between the seeds, with a beak, glabrous, dehiscent with 3 valves. Seeds are globose to ovoid or trigonous, 10–12 mm × 10–12 mm, brown.

Moringa peregrina **seeds are used to produce oil, called 'ben oil'. This oil use goes back to the Faeroes time. It is used for cooking, in cosmetics and in medicine.**

1.6.10.2. Major Chemical Constituents

The seed of Moringa peregrina contains about 50% oil. The approximate fatty acid composition of the oil is: palmitic acid 9%, stearic acid 4%, arachidic acid 2%, behenic acid 2%, oleic acid 71%, linoleic acid 1%, and gadoleic acid 2%. The oil contains the sterols campesterol, stigmasterol and β-sitosterol and the tocopherols α-, γ-, and δ-tocopherol. The water

purifying properties of the seed are caused by a protein which coagulates dispersed particles.

1.6.10.3. Medicinal and Pharmacological Activities

a. Antibacterial Activity

Extracts Moringa peregrina was found to be active against Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans (Marwah et al. 2006).

b. Other Activities

The juice from the bark of Moringa peregrina is used as a disinfectant and to speed up wound healing (Ruchi et al. 2006). The seeds are also used as coagulant to purify water, e.g. in Sudan (Jahn 1986). The oil is used to treat abdominal pain.

1.6.11. Biota orientalis

1.6.11.1. Habitat, Origin and Botanical Description

Biota orientalis Endl (*Thuja orientalis* L.) is a gymnospermic plant of the family *Cupressaceae*, order *Coniferales*. It is known in English as Easern red, and in Arabic as "Althoya" (الثويا) and "Alafssa" (العفصة). Based on morphological and embryological characteristics *Biota orientalis* Endl, which was known as *Thuja orientalis* L. has been separated from the genus *Thuja* and named as *Biota* (Buchholz 1948; Singh, and Oberoi 1962).

Biota orientalis Endl is a popular graceful and beautiful widely cultivated ornamental plant with high medicinal value. It is an evergreen tree with profuse branching reaching a maximum height of 15 m. There is

considerable variation in shape of the tree under cultivation. The stem divides profusely into vertical main branches near the base. The branches are covered with minute scale-like dark green, opposite and decussate, closely adpressed leaves having a long groove in the middle of their abaxial (lower) surface. The leaves are fused with the stem up to one third of their length (Gahalain 2006).

1.6.11.2. Major Chemical Constituents

Biota orientalis Endl is one of the 50 fundamental herbs (Duke and Ayensu 1985) in Chinese herbalism, Both the leaves and the seeds contain an essential oil consisting of borneol, bornyl acetate, thujone, camphor and sesquiterpenes (Nguyen and Doan 1989).

Biota orientalis oil was found to include 3-carene (57.06%), β-pinene (6.90%), limonene (4.28%), cis-thujone (4.16%), cedrol (4.08%), α-terpineol (3.43%), α-pinene (3.19%), δ-2-carene (2.25%) and about 2.18% fenchone (Pandey and Chowdhury 2002).

1.6.11.3. Medicinal and Pharmacological Activities

a. ***Antibacterial Activity***

The lipids of the seeds of Biota orientalis are found to be effective against various microorganisms including Bacillus megaterium, Bacillus brevis, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter aerogenes, Staphylococcus and Micrococcus luteus (Metin et al. 2002).

b. ***Other Activities***

The leaves of *Biota orientalis* used fresh or dry are antibacterial, antipyretic, astringent, diuretic, expectorant, refrigerant, stomachic and

improve hair growth. They are used internally in the treatment of coughs, colds, haemorrhages, excessive menstruation, bronchitis, asthma, skin infections, mumps, bacterial dysentery, arthritic pain and premature baldness. The immature female cones are very effective in the treatment of warts. The seed is an aperient, lenitive and sedative. It is used internally in the treatment of palpitations, insomnia, nervous disorders and constipation in the elderly (Yeung; Brown 1995).

1.6.12. Ferula asafoetida

1.6.12.1. Habitat, Origin and Botanical Description

Ferula asafoetida L. is of the order *Apiales* and the family *Umbelliferae*. It is known in English as Asafoetida and the devil's dung and in Arabic as "Heltit" (حَلْتِيت). It is native to Iran, Arabia and Sudan. *Ferula assafoetida* is herbaceous and monoecious. It grows to 2 m high with a circular mass of leaves. Flowering stems are 2.5–3 m high and 10 cm thick, with a number of schizogenous ducts in the cortex containing the resinous gum. Stem leaves have wide sheathing petioles. Compound large umbels arise from large sheaths. Flowers are pale greenish yellow. Fruits are oval, flat, thin, reddish brown and have a milky juice. Roots are thick, massive, and pulpy. It yields a resin similar to that of the stems. All parts of the plant have the distinctive fetid smell (Ross).

Ferula asafoetida L. has a pungent garlic smell when raw, but in cooked dishes, it delivers a smooth flavour, reminiscent of leeks.

1.6.12.2. Major Chemical Constituents

Dried *Ferula asafoetida L.* consists mostly of a resin (25 to 60% of the total mass, 60% of which are esters of ferula acid) and a complex carbohydrate part (25 to 30%). The essential oil (10%) contains a wealth

of sulfur compounds, mainly (R)-2-butyl-1-propenyl disulphide (50%), 1-(1-methylthiopropyl) 1-propenyl disulphide and 2-butyl-3-methylthioallyl disulphide. Furthermore, di-2-butyl trisulphide, 2-butyl methyl trisulphide, di-2-butyl disulphide and even di-2-butyl tetrasulphide have been found (Rajanikant et al. 1984). The resin portion is known to contain asareninotannols 'A' and 'B', ferulic acid, umbelliferone and four unidentified compounds (Singhal 1997).

1.6.12.3. Medicinal and Pharmacological Activities

Ferula asafoetida L. is traditionally used to treat flatulence and spasmodic contraction of stomach and oesophagus with reverse peristalsis. It has been found to affect favourably deep ulcerations, caries of bones, especially in the syphilitic organism. It is also prescribed for respiratory conditions like asthma, bronchitis and whooping cough.

The of dried root latex powder of *Ferula asafoetida L.* has molluscicidal activity against the snail *Lymnaea acuminate (*Kumar and Singh 2005).

1.7. Panel of Standard Antibiotics

To ensure proper interpretation of the results, four standard antibiotics were used for reference, namely Benzylpenicillin, Erythromycin, Gentamicin and Ceftriaxone. These four antibiotics are commercially available and widely used against the test bacteria.

1.7.1. Benzylpenicillin

Benzylpenicillin, commonly known as penicillin G, is the gold standard penicillin. It is typically given by a parenteral route of administration (not orally) because it is unstable in the hydrochloric acid of the stomach. Because the drug is given parenterally, higher tissue concentrations of

penicillin G can be achieved leading to increased antibacterial activity. Benzylpenicillin is used to treat Cellulitis, Bacterial endocarditis, Gonorrhea, Meningitis, aspiration pneumonia, lung abscess, Community-acquired pneumonia, Syphilis and Septicemia in children (Russell 2002, Katzung).

1.7.1.1. Mechanism of action

β-lactam antibiotics work by inhibiting the formation of peptidoglycan cross-links in the bacterial cell wall. The β-lactam moiety (functional group) of penicillin binds to the enzyme (DD-transpeptidase) that links the peptidoglycan molecules in bacteria, which weakens the cell wall of the bacterium (in other words, the antibiotic causes cytolysis or death due to osmotic pressure). In addition, the build-up of peptidoglycan precursors triggers the activation of bacterial cell wall hydrolases and autolysins, which further digest the bacteria's existing peptidoglycan (Russell 2002, Katzung).

Gram-positive bacteria are called protoplasts when they lose their cell wall. Gram-negative bacteria do not lose their cell wall completely and are called spheroplasts after treatment with penicillin.

Penicillin shows a synergistic effect with aminoglycosides, since the inhibition of peptidoglycan synthesis allows aminoglycosides to penetrate the bacterial cell wall more easily, allowing its disruption of bacterial protein synthesis within the cell. This results in a lowered MBC for susceptible organisms.

1.7.1.2. Clinical use

Penicillin (sometimes abbreviated PCN or pen) is a group of β-lactam antibiotics used in the treatment of bacterial infections caused by

susceptible, usually Gram-positive, organisms. “Penicillin” is also the informal name of a specific member of the penicillin group Penam Skeleton, which has the molecular formula R-C9H11N2O4S, where R is a variable side chain (Rossi 2006, James 2007).

1.7.2. Erythromycin

Erythromycin is a macrolide antibiotic that is similar to but wider than that of penicillin in terms of antimicrobial spectrum. It is often used for people who are allergic to penicillins. It has a good coverage of atypical organisms associated with respiratory tract infections, including mycoplasma and Legionellosis. It is also used to treat chlamydia, syphilis, acne, and gonorrhoea (Russell 2002, Katzung).

1.7.2.1. Mechanism of action

Erythromycin may possess bacteriocidal activity, particularly at higher concentrations (Katzung). The mechanism is not fully elucidated however. By binding to the 50S subunit of the bacterial 70S rRNA complex, protein synthesis and subsequently structure/function processes critical for life or replication are inhibited (Katzung). Erythromycin interferes with aminoacyl translocation, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the P site of the rRNA complex. Without this translocation, the A site remains occupied and thus the addition of an incoming tRNA and its attached amino acid to the nascent polypeptide chain is inhibited. This interferes with the production of functionally useful proteins and is therefore the basis of antimicrobial action (Russell 2002, Katzung).

1.7.2.2. Clinical use

Erythromycin is used to treat (first-line treatment minor wound and skin infection that are likely to be infected with *staphylococci* or *streptococci*. It is also, used as an antibiotic for prophylaxis of wounds at risk of developing infection. It may be appropriate in some circumstances for wounds to the feet, extensive intra-oral lacerations, or stellate lesions (Russell 2002, Katzung).

1.7.3. Gentamicin

Gentamicin is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria, including *Pseudomonas*, *Proteus*, *Serratia*, and Gram-positive *Staphylococcus*. However, it is not used for *Neisseria gonorrhoeae*, *Neisseria meningitidis* or *Legionella pneumophila* bacterial infections (Russell 2002).

1.7.3.1. Mechanism of action

Gentamicin binds to bacterial ribosome and induces misreading of mRNA hence inappropriate amino acid insertions (bacteriocidal). It requires IV/IM administration and poor CSF penetration (unless inflammed meninges). It is filtered unchanged by kidney hence accumulate in renal impairment. Gentamicin is active against aerobic gram-negative organisms and *staphylococci*.

1.7.3.2. Clinical Use

Usually used as part of a combination regimen to treat serious sepsis (septicaemia). It is also, used topically to treat eye and ear infections (Russell 2002).

1.7.4. Ceftriaxone

Ceftriaxone is a third-generation cephalosporin; it has broad spectrum activity against Gram positive and Gram negative bacteria. In most cases, it is considered to be equivalent to cefotaxime in terms of safety and efficacy (Russell 2002).

1.7.4.1. Mechanism of action

The syn-configuration of the methoxyimino moiety confers stability to β-lactamase enzymes produced by many Gram-negative bacteria. Such stability to β-lactamases increases the activity of ceftriaxone against otherwise resistant Gram-negative bacteria. In place of the easily hydrolysed acetyl group of cefotaxime, ceftriaxone has a metabolically-stable thiotriazinedione moiety (Russell 2002).

1.7.4.2. Clinical Use

Ceftriaxone is often used (in combination, but not direct, with macrolide and/or aminoglycoside antibiotics) for the treatment of community-acquired pneumonia. It is also a choice drug for treatment of bacterial meningitis. In pediatrics, it is commonly used in febrile infants between 4 and 8 weeks of age who are admitted to the hospital to exclude sepsis. It has also been used in the treatment of Lyme disease and gonorrhoea (Russell 2002).

1.8. Test Bacterial Organisims

In this study bacteria prevalent in abscesses and wounds were isolated and used to determine their susceptibility to extracts of 12 medicinal plants. And for reference, a panel drug-sensitive reference bacterial strains that belongs to different classes and consist of Gram-positive and Gram-negative bacteria was used.

The bacteria species used were Escherichia coli, Klebsiella spp and Proteus vulgaris of the Enterobacteriaceae group, Staphylococcus aureus of the Staphylococci group, Pseudomonas aeruginosa of the Pseudomonads group, and Bacillus subtilis of the Bacilli group. Characteristics, Classification and diseases caused by bacteria of these groups are reviewed below:

1.8.1. *Enterobacteriaceae*

The *Enterobacteriaceae* are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (eg, *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*,) and others. Some enteric organisms, eg, *Escherichia coli*, are part of the normal flora and incidentally cause disease, while others, the *salmonellae* and *shigellae*, are regularly pathogenic for humans. The *Enterobacteriaceae* are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. *Enterobacteriaceae*, enteric gram-negative rods, and enteric bacteria are the terms used generally, but these bacteria may also be called coliforms (Blair et al 1970, Jawetz, et al 1995).

1.8.1.1. Classification

The taxonomy of the *Enterobacteriaceae* is complex and is rapidly changing as further DNA homology studies are performed. More than 20 genera and 100 species have been defined (Kelly and Farmer 1991).

The family *Enterobacteriaceae* is characterised biochemically by the ability to reduce nitrates to nitrites and to ferment glucose with the production of acid or acid and gas. The *Enterobacteriaceae* do not require increased amounts of sodium chloride for growth and are oxidase-negative. Many biochemical tests are used to differentiate species of *Enterobacteriaceae* (Blair et al 1970, Jawetz, et al 1995).

1.8.1.2. Morphology and Identification

A. Typical Organisms: The *Enterobacteriaceae* are short gram-negative rods that may form chains. Typical morphology is seen in growth on solid media in vitro, but morphology is highly variable in clinical specimens. Capsules are large and regular in *Klebsiella,* less so in *Enterobacter,* and uncommon in the other species (Blair et al 1970, Jawetz, et al 1995).

B. Culture: *Escherichia coli,* and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. *Enterobacter* colonies are similar but somewhat more mucoid. *Klebsiella* colonies are large and very mucoid and tend to coalesce with prolonged incubation. The *salmonellae* and *shigellae* produce colonies similar to *Escherichia coli,* but do not ferment lactose. Some strains of *Escherichia coli,* produce hemolysis on blood agar.

C. Growth Characteristics: Carbohydrate fermentation patterns and the activity of amino acid decarboxylases and other enzymes are used in

biochemical differentiation. Some tests, eg, the production of indole from tryptophan, are commonly used in rapid identification systems, while others, eg. the Voges-Proskauer reaction (production of acetyl methylcarbinol from dextrose), are used less often. Culture on "differential" media that contain special dyes and carbohydrates (eg, eosin-methylene blue [EMB], MacConkey's, or deoxycholate medium distinguishes lactose-fermenting (colored) from non-lactose-fermenting colonies (nonpigmented) and may allow rapid presumptive identification of enteric bacteria (Blair et al 1970, Jawetz, et al 1995).

1.8.1.3. Escherichia coli

Escherichia coli typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *Escherichia coli,* by its hemolysis on blood agar, typical colonial morphology with an iridescent "sheen" on differential media such as EMB agar, and a positive spot indole test. Over 90% *of Escherichia coli,* isolates are positive for (3-glucuronidase using the substrate 4-methylumbelliferyl-P-glu-curonide (MUG). Isolates from anatomic sites other than urine, with characteristic properties (above plus negative oxidase tests) often can be confirmed as *Escherichia coli,* with a positive MUG test (Blair et al 1970, Jawetz, et al 1995).

1.8.1.4. Klebsiella aerogenes

Klebsiella *aero-genes* exhibit mucoid growth, large poly-saccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate. Most *Enterobacter aero-genes* give positive tests for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Enterobacter aero-genes* have small

capsules. *Serratia* produces DNase, lipase, and gelatinase. *Klebsiella, Enterobacter,* and *Serratia* usually give positive Voges-Proskauer reactions (Blair et al 1970, Jawetz, et al 1995).

1.8.1.5. Proteus vulgaris

Proteus vulgaris deaminate phenylalanine, are motile, grow on potassium cyanide medium (KCN), and ferment xylose. *Proteus* ***vulgaris*** move very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystine-lactose-electrolyte-deficient) medium. *Proteus* ***vulgaris*** and *Morganella morganii* are urease-positive, while *Providencia* species usually are urease-negative. The *Proteus-Providencia* group ferment lactose very slowly or not at all. *Proteus mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group (Blair et al 1970, Jawetz, et al 1995).

1.8.1.6. Colicins (Bacteriocins), Toxins and Enzymes

Many gram-negative organisms produce bacteriocins. These virus-like bactericidal substances are produced by certain strains of bacteria active against some other strains of the same or closely related species. Their production is controlled by plasmids. Colicins are produced by *Escherichia coli,* marcescins by *Serratia,* and pyocins by *Pseudomonas.* Bacteriocin-producing strains are resistant to their own bacteriocin; thus, bacteriocins can be used for "typing" of organisms (Blair et al 1970, Jawetz, et al 1995).

Most gram-negative bacteria possess complex lipopolysaccharides in their cell walls. These substances, endotoxins, have a variety of

pathophysiologic effects. Many gram-negative enteric bacteria also produce exotoxins of clinical importance.

1.8.1.7. Diseases Caused by Enterobacteriaceae

1.8.1.7.1. Causative Organisms

Escherichia coli is a member of the normal intestinal flora. Other enteric bacteria *(Proteus, Enterobacter, Klebsiella, Morganella* species) are also found as members of the normal intestinal flora but are considerably less common than *Escherichia coli.* The enteric bacteria are sometimes found in small numbers as part of the normal flora of the upper respiratory and genital tracts. The enteric bacteria generally do not cause disease, and in the intestine they may even contribute to normal function and nutrition. When clinically important infections occur, they are usually caused by *Escherichia coli,* but the other enteric bacteria are causes of hospital-acquired infections and occasionally cause community-acquired infections. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora sites. The most frequent sites of clinically important infection are the urinary tract, biliary tract, and other sites in the abdominal cavity, but any anatomic site (eg, bacteremia, prostate gland, lung, bone, meninges) can be the site of disease. Some of the enteric bacteria are opportunistic pathogens. When normal host defenses are inadequate—particularly in infancy or old age, in the terminal stages of other diseases, after immunosuppression, or with in-dwelling venous or urethral catheters—localized clinically important infections can result, and the bacteria may reach the blood stream and cause sepsis (Blair et al 1970, Jawetz, et al 1995).

a. Escherichia coli

Urinary tract infection- ***Escherichia coli*** **is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection. None of these symptoms or signs is specific for** ***Escherichia coli*** **infection. Urinary tract infection can result in bacteremia with clinical signs of sepsis (Blair et al 1970, Jawetz, et al 1995).**

Nephropathogenic- Escherichia coli typically produce a hemolysin. Most of the infections are caused by Escherichia coli with a small number of 0 antigen types. K antigen appears to be important in the pathogenesis of upper tract infection. Pyelonephritis is associated with a specific type of pilus, P pilus, which binds to the P blood group substance.

A. ***Escherichia coli*** **associated diarrheal diseases- Escherichia coli that cause diarrhea are extremely common worldwide. These Escherichia coli are classified by the characteristics of their virulence properties (see below), and each group causes disease by a different mechanism..**

B. **Sepsis- When normal host defenses are inadequate;** ***Escherichia coli*** **may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to** ***Escherichia coli*** **sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infections.**

C. **Meningitis-** ***Escherichia coli*** **and group B streptococci are the leading causes of meningitis in infants. Approximately 75% of Escherichia coli from meningitis cases have the KI antigen. This antigen cross-reacts with the group B capsular polysaccharide of N meningitidis. The**

mechanism of virulence associated with the KI antigen is not understood.

a. Klebsiella spp

The pathogenesis of disease caused by these groups of enteric gram-negative rods is similar to that of the nonspecific factors in disease caused by *Escherichia coli.*

Klebsiella *pneumoniae* is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (about 3%) of bacterial pneumonias. ***Klebsiella pneumoniae*** can produce extensive hemorrhagic necrotising consolidation of the lung. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients. Other enterics also may produce pneumonia. ***Klebsiella pneumoniae*** and ***Klebsiella oxytoca*** cause hospital-acquired infections. Two other *klebsiellae* are associated with inflammatory conditions of the upper respiratory tract: ***Klebsiella ozaenae*** has been isolated from the nasal mucosa in ozena, a fetid, progressive atrophy of mucous membranes: and ***Klebsiella rhinoscleromatis*** from rhinoscleroma, a destructive granuloma of the nose and pharynx (Blair et al 1970, Jawetz, et al 1995).

c. Proteus spp

Proteus-Proteus species produce infections in humans only when the bacteria leave the intestinal tract. They are found in urinary tract infections and produce bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving intravenous infusions. *Proteus mirabilis* causes urinary tract infections and occasionally other

infections. *Proteus vulgaris* and *Morganella morganii* are important nosocomial pathogens.

1.8.1.8. Treatment

No single specific therapy is available. The sulfon-amides, ampicillin, cephalosporins, chloramphenicol, tetracyclines, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential. Multiple drug resistance is common and is under the control of transmissible plasmids.

1.8.1.9. Epidemiology, Prevention and Control

The enteric bacteria establish themselves in the normal intestinal tract within few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora. *Escherichia coli* is the prototype. Enterics found in water or milk is accepted as proof of fecal contamination from sewage or other sources (Blair et al 1970, Jawetz, et al 1995).

Control measures are not feasible as far as the normal endogenous flora is concerned. Enteropathogenic *Escherichia coli* serotypes should be controlled like salmonellae. Some of the enterics constitute a major problem in hospital infection. It is particularly important to recognise that many enteric bacteria are "opportunists" which cause illness when they are introduced into debilitated patients. Within hospitals or other institutions, these bacteria commonly are transmitted by personnel, instruments, or parenteral medications. Their control depends on hand washing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the

urinary tract sterile (ie, closed drainage) (Blair et al 1970, Jawetz, et al 1995).

1.8.2. Staphylococci

The *Staphylococci* are gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic *Staphylococci* often hemolyse blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable *staphylococcai* enterotoxin. *Staphylococci* rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems (Blair et al 1970, Jawetz, et al 1995).

The genus *Staphylococcus* has at least 30 species. The three main species of clinical importance are *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus saprophyticus. Staphylococcus aureus* is coagulase-positive, which differentiates it from the other species. *Staphylococcus aureus.* is a major pathogen for humans. Almost every person will have some type of *Staphylococcus aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative *Staphylococci* are normal human flora and sometimes cause infection, often associated with implanted appliances and devices, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by coag-ulase-

negative *Staphylococci* are due to *S. epidermidis;* infections due to *Staphylococcus warneri, Staphylococcus hominis,* and other species are less common. *S. saprophyticus* is a relatively common cause of urinary tract infections in young women. Other species are important in veterinary medicine (Blair et al 1970, Jawetz, et al 1995).

1.8.2.1. Morphology and Identification

Typical Organisms: *Staphylococci* are spherical cells about 1 μm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. *Staphylococci* are nonmotile and do not form spores. Under the influence of drugs like penicillin, *Staphylococci* are lysed (Blair et al 1970, Jawetz, et al 1995).

Culture: *Staphylococci* grow readily on most bacteriologic media under aerobic or microaerophilic conditions. They grow most rapidly at 37 °C but form pigment best at room temperature (20-25 °C). Colonies on solid media are round, smooth, raised, and glistening. S aureus usually forms grey to deep golden yellow colonies. *Staphylococcus epidermidis* colonies usually are grey to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of hemolysis are produced by *Staphylococcus aureus* and occasionally by other species *(*Blair et al 1970, Jawetz, et al 1995).

Growth Characteristics: The *Staphylococci* produce catalase, which differentiates them from the *streptococci*. *Staphylococci* slowly ferment many carbohydrates including lactose, producing lactic acid but not gas. Proteolytic activity varies greatly from one strain to another. Pathogenic

Staphylococci **produce many extracellular substances (Blair et al 1970, Jawetz, et al 1995).**

Staphylococci **are relatively resistant to drying, heat (they withstand 50 °C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene.** ***Staphylococci*** **are variably sensitive to many antimicrobial drugs. Resistance falls into several classes (Blair et al 1970, Jawetz, et al 1995):**

(1) β-lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins (penicillin G, ampicillin, ticarcillin, and similar drugs). The plasmids are transmitted by-transduction and perhaps also by conjugation.

(2) Resistance to nafcillin (and to methicillin and oxacillin) is independent of β -lactamase production. The genes for nafcillin resistance reside on the chromosome and are variably expressed. The mechanism of nafcillin resistance is related to the lack or inaccessibility of certain penicillin-binding proteins (PBPs) in the organisms.

(3) "Tolerance" implies that ***Staphylococci*** **inhibited by a drug but not killed by it, i.e., there is very large difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug. Tolerance can at times be attributed to a lack of activation of autolytic enzymes in the cell wall.**

(4) Plasmids can also carry genes for resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs. Staphylococci have remained susceptible to vancomycin (Blair et al 1970, Jawetz, et al 1995).

D. Variation: A culture of *Staphylococci* contains some bacteria that differ from the bulk of the population in expression of colony characteristics (colony size, pigment, hemolysis), in enzyme elaboration, in drug resistance, and in pathogenicity. *In vitro*, the expression of such characteristics is influenced by growth conditions: When nafcillin-resistant *S*. aureus is incubated at 37 °C on blood agar, one in 10^7 organisms expresses nafcillin resistance; when it is incubated at 30 °C on agar containing 2-5% sodium chloride, one in 10^3 organisms expresses nafcillin resistance (Blair et al 1970, Jawetz, et al 1995).

1.8.2.2. Toxins and Enzymes

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzyme (Catalase; Coagulase, hyaluronidase; staphylokinase proteinases; lipases; and β-lactamase). Others are considered to be toxins (Exotoxins; Leukocidin; Exfoliative; Toxic Shock Syndrome Toxin; and Enterotoxins) (Blair et al 1970, Jawetz, et al 1995).

1.8.2.3. Diseases Caused by Staphylococci

Staphylococci, particularly *Staphylococcus epidermidis,* are members of the normal flora of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *Staphylococcus aureus* occurs in 40-50% of humans. *Staphylococci* are also found regularly on clothing, bed linens, and other fomites in human environments.

The pathogenic capacity of a given strain of Staphylococcus aureus is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is

staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs. The potential contribution of the various extracellular substances in pathogenesis is evident from the nature of their individual actions (Blair et al 1970, Jawetz, et al 1995).

Pathogenic, invasive *Staphylococcus. aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive *Staphylococci* such as *Staphylococcus epidermidis* are coagulase-negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. *Staphylococcus saprophyticus* is typically nonpigmented, novobiocin-resistant, and nonhemolytic; it causes urinary tract infections in young women (Blair et al 1970, Jawetz, et al 1995).

The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Groups of *Staphylococcus aureus* established in a hair follicle lead to tissue necrosis (dermonecrotic factor). Coagulase is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue. Within the center of the lesion, liquefaction of the necrotic tissue occurs (enhanced by delayed hypersensitivity), and the abscess "points" in the direction of least resistance. Drainage of the liquid center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing *(*Blair et al 1970, Jawetz, et al 1995).

Focal suppuration (abscess) is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and

bloodstream to other parts of the body. Suppuration within veins, associated with thrombosis, is a common feature of such dissemination. In osteomyelitis, the primary focus of *S. aureus* growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration. *Staphylococcus aureus* may cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. *Staphylococci* of low invasiveness are involved in many skin infections (eg, acne, pyoderma, or impetigo). Anaerobic cocci *(Peptostrep tococcus)* participate in mixed anaerobic infections (Blair et al 1970, Jawetz, et al 1995).

Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection. Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxin. Toxic shock syndrome is associated with toxic shock syndrome toxin-1 (TSST-1) (Blair et al 1970, Jawetz, et al 1995).

A localised staphylococcal infection appears as a "pimple," hair follicle infection, or abscess. There is usually an intense, localised, painful inflammatory reaction that undergoes central suppuration and heals quickly when the pus is drained. The wall of fibrin and cells around the core of the abscess tends to prevent spread of the organisms and should not be broken down by manipulation or trauma (Blair et al 1970, Jawetz, et al 1995).

***Staphylococcus aureus* infection can also result from direct contamination of a wound, eg, postoperative staphylococcal wound infection or infection following trauma (chronic osteomyelitis subsequent to an open fracture, meningitis following skull fracture).**

If *Staphylococcus aureus* disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other blood stream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration (Blair et al 1970, Jawetz, et al 1995).

Food poisoning due to staphylococcal enterotoxin is characterised by a short incubation period (1-8 hours' violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.

Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases. It often occurs within 5 days after the onset of menses in young women who use tampons, but it also occurs in children or in men with staphylococcal wound infections. The syndrome can recur. Toxic shock syndrome-associated *Staphylococcus aureus* can be found in the vagina, on tampons in wounds or other localised infections, or in the throat but virtually never in the bloodstream (Blair et al 1970, Jawetz, et al 1995).

1.8.2.4. Treatment

Most persons harbor *staphylococci* on the skin and in the nose or throat. Even if the skin can be cleared of *staphylococci* (eg, in eczema), reinfection by droplets will occur almost immediately. Because pathogenic organisms are commonly spread from one lesion (eg, a furuncle) to other areas of the skin by fingers and clothing, scrupulous

local antisepsis is important to control recurrent furunculosis (Blair et al 1970, Jawetz, et al 1995).

Serious multiple skin infections (acne, furunculosis) occur most often in adolescents. Similar skin infections occur in patients receiving prolonged courses of corticosteroids. In acne, lipases of *staphylococci* and corynebacteria liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long-term treatment (Blair et al 1970, Jawetz, et al 1995).

Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy. Many antimicrobial drugs have some effect against *staphylococci in vitro*. However, it is difficult to eradicate pathogenic *staphylococci* from infected persons, because the organisms rapidly develop resistance to many antimicrobial drugs and the drugs cannot act in the central necrotic part of a suppurative lesion. It is also difficult to eradicate the *S. aureus* carrier state (Blair et al 1970, Jawetz, et al 1995).

Acute hematogenous osteomyelitis responds well to antimicrobial drugs. In chronic and recurrent osteomyelitis, surgical drainage and removal of dead bone is accompanied by long-term administration of appropriate drugs, but eradication of the infecting *staphylococci* is difficult. Hyperbaric oxygen and the application of vascularised myocutaneous flaps have aided healing in chronic osteomyelitis (Blair et al 1970, Jawetz, et al 1995).

Bacteremia, endocarditis, pneumonia, and other severe infections due to *Staphylococcus aureus* require prolonged intravenous therapy with a (β-lactamase-resistant penicillin. Vancomycin is often reserved for use with

nafcillin-resistant *staphylococci*. If the infection is found to be due to non-(β-lactamase-producing *S. aureus,* penicillin G is the drug of choice, but only a small percentage of *Staphylococcus aureus* strains are susceptible to penicillin G (Blair et al 1970, Jawetz, et al 1995).

***Staphylococcus epidermidis* infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves from the circulation and thus from antimicrobial drugs. *Staphylococcus epidermidis* is more often resistant to antimicrobial drugs than is *Staphylococcus aureus;* approximately 75% *of Staphylococcus epidermidis* strains are nafcillin-resistant (Blair et al 1970, Jawetz, et al 1995).**

Because of the frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs. Resistance to drugs of the erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection. Drug resistance (to penicillins, tetracyclines, aminoglycosides, erythromycins, etc) determined by plasmids can be transmitted among *staphylococci* by transduction and perhaps by conjugation (Blair et al 1970, Jawetz, et al 1995).

Penicillin G-resistant *Staphylococcus aureus* strains from clinical infections always produce penicillinase. They now constitute about 90% *of Staphylococcus aureus* isolates in communities in the USA. They are often susceptible to lactamase-resistant penicillins, cephalosporins, or vancomycin. Nafcillin resistance is independent of β-lactamase production, and its clinical incidence varies greatly in different countries and at different times. The selection pressure of (β-lactamase-resistant

antimicrobial drugs may not be the sole determinant for resistance to these drugs: For example, in Denmark, nafcillin-resistant *S. aureus* comprised 40% of isolates in 1970 and only 10% in 1980, without notable changes in the use of nafcillin or similar drugs. In the USA, nafcillin-resistant *S. aureus* accounted for only 0.1% of isolates in 1970 but in the 1990s constituted 10-30% of isolates from infections in some hospitals. Vancomycin remains the most widely effective drug against *staphylococci* (etal 1970, Jawetz, et al 1995).

1.8.2.5. Epidemiology and Control

Staphylococci are ubiquitous human parasites. The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant *staphylococci* in the nose or on the skin. Although cleanliness, hygiene, and aseptic management of lesions can control the spread of *staphylococci* from lesions, few methods are available to prevent the wide dissemination of *staphylococci* from carriers. Aerosols (eg, glycols) and ultraviolet irradiation of air have little effect (Blair et al 1970, Jawetz, et al 1995).

In hospitals, the areas at highest risk for severe staphylococcal infections are the newborn nursery, intensive care units, operating rooms, and cancer chemotherapy wards. Massive introduction of "epidemic" pathogenic *Staphylococcus aureus* into these areas may lead to serious clinical disease. Personnel with active *Staphylococcus aureus* lesions and carriers may have to be excluded from these areas. In such individuals, the application of topical antiseptics (eg,

chlorhexidine or bacitracin cream) to nasal or perineal carriage sites may diminish shedding of dangerous organisms. Rifampin coupled with a second oral antistaphylococcal drug sometimes provides long-term suppression and possibly cure of nasal carriage; this form of therapy is usually reserved to major problems of staphylococcal carriage, because *staphylococci* can rapidly develop resistance to rifampin. Antiseptics such as hexachlorophene used on the skin of newborns diminish colonisation by *staphylococci*, but toxicity prevents their widespread (Blair et al 1970, Jawetz, et al 1995).

1.8.3. Pseudomonas Group

The *Pseudomonas* group are gram-negative, motile, aerobic rods some of which produce water-soluble pigments. *Pseudomonads* occur widely in soil, water, plants, and animals. *Pseudomonas aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other *Pseudomonas* species infrequently cause disease. The classification of *pseudomonads* is based on RNA/DNA homology and common culture characteristics (Blair et al 1970, Jawetz, et al 1995).

Pseudomonas aeruginosa is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonise normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defences (Blair e tal 1970, Jawetz, et al 1995).

1.8.3.1. Morphology and Identification

A. Typical Organisms: *Pseudomonas aeruginosa* is motile and rod-shaped, measuring about 0.6 X 2 μm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains.

B. Culture: *Pseudomonas aeruginosa* is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like odor. Some strains hemolyse blood. *Pseudomonas aeruginosa* forms smooth round colonies with a fluorescent greenish color. It often produces the nonfluorescent bluish pigment pyocyanin, which diffuses into the agar. Other *Pseudomonas* species do not produce pyocyanin. Many strains of *Pseudomonas aeruginosa* also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar. Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin.

Pseudomonas aeruginosa in a culture can produce multiple colony types, giving the impression of a culture of mixed species of bacteria. *Pseudomonas aeruginosa* from different colony types may also have different biochemical and enzymatic activities and different antimicrobial susceptibility patterns. Cultures from patients with cystic fibrosis often yield *Pseudomonas aeruginosa* organisms that form very mucoid colonies as a result of overproduction of alginate, an exopolysaccharide.

C. Growth Characteristics: *Pseudomonas aeruginosa* grows well at 37-42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species. It is oxidase-positive. It does not ferment carbohydrates, but many strains oxidise glucose. Identification is usually based on colonial morphology, oxidase positivity, the presence of characteristic pigments, and growth at 42 °C. Differentiation of *Pseudomonas aeruginosa* from other *pseudomonads* on the basis of biochemical activity requires testing with a large battery of substrates (Blair et al 1970, Jawetz, et al 1995).

1.8.3.2. Enzymes and Toxins

Many strains of *Pseudomonas aeruginosa* produce exotoxin A, which causes tissue necrosis and is lethal for animals when injected in purified form. The toxin blocks protein synthesis by a mechanism of action identical to that of diphtheria toxin, though the structures of the two toxins are not identical. Antitoxins to exotoxin A are found in some human sera, including those of patients who have recovered from serious *Pseudomonas aeruginosa* infections (Blair et al 1970, Jawetz, et al 1995).

1.8.3.3. Pathogenesis and Clinical Findings

***Pseudomonas aeruginosa* is pathogenic only when introduced into areas devoid of normal defenses, eg, when mucous membranes and skin are disrupted by direct tissue damage; when intravenous or urinary catheters are used; or when neutropenia is present, as in cancer chemotherapy. The bacterium attaches to and colonises the mucous membranes or skin, invades locally, and produces systemic disease. These processes are promoted by the pili, enzymes, and toxins described above. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria, leukocytosis and leukopenia, disseminated intravascular coagulation, and adult respiratory distress syndrome (Blair et al 1970, Jawetz, et al 1995).**

***Pseudomonas aeruginosa* (and other species, eg, *Pseudomonas cepacia, Pseudomonas putida)* is resistant to many antimicrobial agents and therefore becomes dominant and important when more susceptible bacteria of the normal flora are suppressed.**

***Pseudomonas aeruginosa* produces infection of wounds and burns, giving rise to blue-green pus; meningitis, when introduced by lumbar**

puncture; and urinary tract infection, when introduced by catheters and instruments or in irrigating solutions. Involvement of the respiratory tract, especially from contaminated respirators, results in necrotising pneumonia. The bacterium is often found in mild otitis extema in swimmers. It may cause invasive (malignant) otitis extema in diabetic patients. Infection of the eye, which may lead to rapid destruction of the eye, occurs most commonly after injury or surgical procedures. In infants or debilitated persons, *Pseudomonas aeruginosa* may invade the bloodstream and result in fatal sepsis; this occurs commonly in patients with leukemia or lymphoma who have received antineoplastic drugs or radiation therapy and in patients with severe burns. In most *Pseudomonas aeruginosa* infections, the symptoms and signs are nonspecific and are related to the organ involved. Occasionally, verdoglobin (a breakdown product of hemoglobin) or fluorescent pigment can be detected in wounds, burns, or urine by ultraviolet fluorescence. Hemorrhagic necrosis of skin occurs often in sepsis due to *Pseudomonas aeruginosa;* the lesions, called ecthyma gangrenosum, are surrounded by erythema and often do not contain pus. *Pseudomonas aeruginosa* can be seen on Gram-stained specimens from ecthyma lesions, and cultures are positive. *Ecthyma gangrenosum* is uncommon in bacteremia due to organisms other than *Pseudomonas aeruginosa* (Blair et al 1970, Jawetz, et al 1995).

1.8.3.4. Treatment

Clinically significant infections with *Pseudomonas aeruginosa* should not be treated with single-drug therapy, because the success rate is low with such therapy and because the bacteria can rapidly develop resistance when single drugs are employed. A penicillin active against *Pseudomonas aeruginosa* —ticarcillin, mezlocillin, and piperacillin—is

used in combination with an aminoglycoside, usually gentamicin, tobramycin, or amikacin. Other drugs active against *Pseudomonas aeruginosa* include aztreonam, imipenem, and the newer quinolones, including ciprofloxacin. Of the newer cephalosporins, ceftazidime and cefoperazone are active against *Pseudomonas aeruginosa;* ceftazidime is used in primary therapy of *Pseudomonas aeruginosa* infections. The susceptibility patterns of *Pseudomonas aeruginosa* vary geographically, and susceptibility tests should be done as an adjunct to selection of antimicrobial therapy (Blair et al 1970, Jawetz, et al 1995).

1.8.3.5. Epidemiology and Control

Pseudomonas aeruginosa is primarily a nosocomial pathogen, and the methods for control of infection are similar to those for other nosocomial pathogens. Since *Pseudomonas* thrives in moist environments, special attention should be paid to sinks, water baths, showers, hot tubs, and other wet areas. For epidemiologic purposes, strains can be typed by pyocins and by lipopolysaccharide immunotypes. Vaccine from appropriate types administered to high-risk patients provides some protection against *Pseudomonas* sepsis. Such treatment has been used experimentally in patients with leukemia, burns, cystic fibrosis, and immunosuppression (Blair et al 1970, Jawetz, et al 1995).

1.8.4. Bacillus Species

The genus *Bacillus* includes large aerobic, gram-positive rods occurring in chains. Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus cereus* and *Bacillus subtilis.* Some are insect pathogens. *Bacillus cereus* can grow in foods and produce an enterotoxin or an emetic toxin and cause food poisoning. Such organisms may occasionally produce dis-

ease in immunocompromised humans (eg, meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis). *Bacillus anthracis,* which causes anthrax, is the principal pathogen of the genus (Blair et al 1970, Jawetz, et al 1995).

1.8.4.1. Morphology and Identification

A. Typical Organisms: The typical cells, measuring 1 X 3-4 μm, have square ends and are arranged in long chains; spores are located in the center of the nonmotile bacilli.

B. Culture: Colonies of *Bacillus anthracis* are round and have a "cut glass" appearance in transmitted light. Hemolysis is uncommon with *Bacillus anthracis* but common with the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

C. Growth Characteristics: The saprophytic bacilli utilize simple sources of nitrogen and carbon for energy and growth. The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized only by autoclaving (Blair et al 1970, Jawetz, et al 1995).

1.8.4.2. Bacillus Cereus

Food poisoning caused by Bacillus cereus has two distinct forms, the emetic type and the diarrheal type associated with meat dishes and

sauces. Bacillus cereus produces toxins that cause disease which is more an intoxication than a food-home infection. The emetic form is manifested by nausea, vomiting, abdominal cramps, and occasionally diarrhea and is self-limiting, with recovery occurring within 24 hours. It begins 1-5 hours after ingestion of rice and occasionally pasta dishes. B cereus is a soil organism that commonly con-laminates rice. When large amounts of rice are cooked and allowed to cool slowly, the B cereus spores germinate and the vegetative cells produce the toxin during log-phase growth or during sporulation. The diarrheal form has an incubation period of 1-24 hours and is manifested by profuse diarrhea with abdominal pain and cramps; fever and vomiting are uncommon. The enterotoxin may be preformed in the food or produced in the intestine. The presence of B cereus in a patient's stool is not sufficient to make a diagnosis of B cereus disease, since the bacteria may be present in normal stool specimens; a concentration of 105 bacteria or more per gram of food is considered diagnostic (Blair et al 1970, Jawetz, et al 1995).

Bacillus cereus **is an important cause of eye infections, severe keratitis, endophthalmitis, and panophthalmitis. Typically, the organisms are introduced into the eye by foreign bodies associated with trauma.** ***B cereus*** **has also been associated with localized infections and with systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections.**

Other ***Bacillus*** **species are rarely associated with human disease. It is difficult to differentiate superficial contamination with** ***Bacillus*** **from genuine disease caused by the organism. Five** ***Bacillus*** **species** ***(Bacillus***

thuringiensis, Bacillus popilliae, Bacillus sphaericus, Bacillus larvae, and *Bacillus lentifnorbus)* are pathogens for insects, and some have been used as commercial insecticides (Blair et al 1970, Jawetzet al. 1995).

1.8.5. Methicillin-resistant Staphylococcus aureus (MRSA)

Enteric bacterial infection caused by resistant strains of *Staphylococcus aureus* , *Eschrichia coli*, *Shigella* and other bacteria are becoming more and more problematic and of major public health problem in both developed and developing countries (Livermore, 1995, Westh et al., 2004). Infections with MRSA occur due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases and due to the poor hygienic conditions in both hospital and community, which is more prevalent in developing countries. These infections in many cases lead needlessly to deaths especially among the elderly. These deaths lead to huge bills of compensations that financially cripple hospitals (Parekh, and Chanda 2007).

In the past, mutation was the only known cause of resistance of bacteria to drugs. However, it was reported that multi-drug-resistant pathogens are on the rise, which was attributed to plasmid-mediated (Jones et al., 2004), where β-lactamases continues to be the leading cause of resistance to β-lactam antibiotics in Gram- positive bacteria (*Staphylococcus aureus* and vancomycin resistant enterococci) and Gram- negative bacteria (members of enterobacteriaceae producing plasmid-mediated extended spectrum β-lactamase (ESbL)) (Bradford, 2001). Resistance to β-lactam antibiotics was observed in other bacteria including *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*

(Medeiros, 1997; Sajduda et al., 1998), *Eschrichia coli* and other members of *enterobacteriaceae* (Wang et al., 2003; Cheung et al., 2005). Therefore, search for new antimicrobials to combat infectious diseases caused by multidrug-resistant bacteria including fast-spreading ESbL-producing enteric bacteria is urgently needed.

Chapter Two

Materials and Moethods

2. Materials and Methods

2.1. Materials

Chemicals, reagents, chemotherapeutic agents, Culture media, Equipment and Instruments and Bacterial micro-organisms used in this study and their suppliers are itemised in the tables bellow:

Table 1: Chemicals and Reagents

Chemical	Supplier
Amyl Alcohol	The British Drug House UK
Chloroform	S.D. Fine-Chem. Ltd., Mumbai
Crystal violet	The British Drug House UK
D (-) Glucose anhydrous	Park, Scientific Ltd., UK
D (4-) Lactose	Park, Scientific Ltd., UK
D (-) Mannitol extra pure	Kiran Light Lab.
Ethylenediamine tetra-acetic acid	The British Drug House, UK
Emulsifying wax	Medex, UK
Halothane	ICI Ltd., India
Hydrochloric acid	The British Drug House, UK
Hydrogen peroxide	Bell, sons & Co., UK
Immersion oil	The British Drug House, UK
Iodine	Hopkins & Williams Ltd.
Kovac's reagent	B.G.H Chemicals, Japan
Lactophenol cotton blue	The British Drug House, UK
LanolineB.P.	Sukani Enterprises, India
Liquid paraffin BP	Bell, sons & Co., UK
Methanol	Scharlu, Spain
Methyl Red	Oxoid, Ltd.
Oxidase	John baker Inc., USA
Peptone	Oxoid, Ltd.
Petroleum Jelly B.P.	Bogdany, Hungary
Phenol Red	The British Drug House, UK
Polyethylene glycol (PEG)	Flukp AG Chemische Fabrik
Safranin Red	The British Drug House, UK
Sodium Chloride	The British Drug House, UK
Sucrose AR	S.D. Fine-Chem. Ltd., Mumbai
Urea	Oxoid, Ltd.
Petroleum Ether	The British Drug House, UK
Benzene	The British Drug House, UK

Table 2: Chemotherapeutic agents

Agent	Batch No	Supplier
Benzylpenicillin	111472E	Alembic Ltd, India
Gentamycin	061108	Shaphar. Shanghai, China
Ceftriaxone	KF-610	Kilitch Drugs (India) Ltd. Thane, India
Erythromycin		xxxx

Table 3: Culture media

Culture	Supplier
Blood	Khartoum Hospital, Sudan
Blood agar base	Mast group Ltd., UK
DNase media	Plasmatec Lab Ltd., UK
K.LA. media	Mast group Ltd., UK
Koser citrate medium	Oxoid Ltd.
Mac Conkey agar	Scharlu, Spain
Mannitol Salt agar	Oxoid Ltd.
Nutrient agar	Scharlu, Spain
Nutrient broth	Scharlu, Spain
Sabouraud Dextrose agar	Plasmatec Lab. Ltd., UK
Urea agar base	Oxoid Ltd.

Table 4: Equipment and Instruments

Equipment	Supplier
Autoclave	Baird & Tatlock Ltd.
Balance	A & D Company Ltd.
Centrifuge	Braun, Centrifuge PLC series
Colony counter	Anderman colony counter, UK.
Glass ware	Griffin & George Ltd.
Hot air oven	Nuve FN 500, Turkey
Incubator	Heraeus
Microscope	Olympus CH-2, Japan
Rota-vap	Buchi, Switzerland
Soxhiet	Grant Instruments Ltd.
Water bath	Fisher scientific, USA

Table 5: Bacterial micro-organisms

Bacterial organism	Code
Bacillus subtilis	NCTC 8236
Staphylococcus aureus	ATCC 25923
Escherichia coli	ATCC 25922
Pseudomonas aeruginosa	ATCC 27853
Klebsiella spp	ATCC 35657

Table 6: Clinical Bacterial Organisms from Wounds and Abscesses of Adult Patients (Khartoum Hospital, Sudan)

Bacterial Oraganism	Numbers (%)
Escherichia coli	20
Pseudomonas aeruginosa	20
Proteus vulgaris	18
Klebseilla sp.	17
Staphylococcus aureus	18
Methicillin-resistant *Staphylococcus aureus (MRSA)*	7

Table 7: Medicinal Plants selected for the study

Latin name	*Arabic name*	*English name*
Piper cubeba	حب العروس , كَبَابَة	Jawa peppercorn
Anogussus leiocapus	الصحب	Axlewood tree
Biota orientalis	الثويا, العفصة	Eastern red
Punica granatum (Bark)	الرمان	Pomegranate
Punica granatum (Wood)	الرمان	Pomegranate
Calendula arvensis	الحنوه	Field marigold
Salvia officinalis	مريمية	Garden sage
Nauclia latifolia	كرمدودة	Pin cushion tree
Cissus petiolata	عرق الحجر	Bitter Aple and Bitter Groud
Petasties hybridus L.	فكهت	Butterbur
Acorus calamus	الذر يره	Sweet Flag
Ferula Asafoetida	الحَلتيت	devil's dung
Moringa perigrina	اليسر، شجرة الراواق	Drum-stick tree

2.2. Methods

2.2.1. Plant materials

The 12 medicinal plants used in this study, their botanical names, synonyms, families, morphological parts used, vernacular names and uses are shown in Table 7. These plants were obtained from local herbalists, Omdurman and Khartoum Souks (Markets). Local herbalists provided useful information including the description of the studied plants habitat, their local names, traditional method of preparing the herbal potions, the ailments they claim to cure and the methods of their applications.

The plants were identified and authenticated by the Department of Botany, University of Khartoum and the Medicinal and Aromatic Plants Research Institute.

Ten grams sample of each of the 12 studied plants (Table 7) were taken, powdered and extracted and then they were screened for their antibacterial activity.

2.2.2. Preparation of the crude extracts

a. Continuous extraction

Each of the powdered plant material samples (10 g) was extracted by Benzene, Chloroform and Methanol consecutively. Firstly, the plant material sample was exhaustively extracted by benzene in a Soxhlet apparatus. The process took in average 24 hours. The benzene extracts were filtered and evaporated under reduced pressure using Rota-vap. The residue was weighed and the yield percentage was determined. The extracted plant materials were then air-dried, The extracted and air-dried plant materials were then, placed in the Soxhlet and exhaustively extracted with Chloroform. The extract was filtered and evaporated under reduced pressure again using Rota-vap. The residue was weighed and the yield percentage was determined. The extracted plant materials were then air-dried,

The Chloroform extracted and air-dried plant materials were then extracted with Methanol adopting the method described above. The residue was weighed and the yield percentage was determined.

b. Maceration

The powdered plant materials (10g) were macerated separately by immersion for 2 hours in 3:7 water and methanol. The extracts were filtered and evaporated under reduced pressure using Rota-vap. The residue was then dried and weighed and the yield percent was obtained. Also, the extracted plant materials were air-dried and then weighed and the yield percentage was determined.

2.2.3. Phytochemical screening

A general screening was performed to identify the chemical constituent of the plants using the following method (Harbone and Baxter 1995)

2.2.3.1. Preparation of the extract

Samples of the powdered part of the plants (10 grams) were refluxed with 100 ml of 80% ethanol in a round bottle flask for 4 hours. The cool solution was filtered and 80% ethanol was added to complete the filtrate to 100 ml. The extracts prepared were used for the identification of the chemical constituent of the plants.

2.2.3.2. Testing for the presence of Unsaturated Sterols and Triterpenses

A sample (10 ml) of each extract prepered as above was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml portion of the chloroform solution was mixed with .0.5 ml of acetic anhydride followed by 2 drops of concentrated sulphuric acid .The gradual

appearance of green, blue & pink to purple colour was taken as an evidence for the presence of sterols (green to blue) and Triterpenses (pink to purple) in the sample .

2.2.3.3. Testing for the presence of Alkaloids

A sample (7.5 ml) of each extract prepared as above was evaporated to dryness on a water bath. 5 ml of 2N HCL was added and stirred while heating on the water bath for 10 minutes , and then cooled , filtered and divided into two test tube .To first tube few drops of Mayor's reagent were added to it, while to the other tube few drops of Valser's reagent were added to it. A slight turbidity as heavy precipitate in either of the two test tubes was taken as a presumptive evidence for the presence of alkaloids.

2.2.3.4. Testing for the presence of Flavonoids

A sample (7.5 ml) of extract prepared as above was evaporated in a water bath, cooled and the residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in 30 ml of 80% ethanol and filtered. To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds.

2.2.3.5. Testing for the presence of Tannins

A sample (7.5 ml) of each of the extracts prepared was evaporated to dryness on water bath. The residue was extracted several times with N-hexane and filtrated. The insoluble residue was stirred with 10 ml of hot saline solution. The mixture was cooled and filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. The formation

of immediate precipitate was taken as evidence for the presence of tannin in the plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added .The formation of blue, black or green colour was taken as an evidence for the presence of tannins.

2.2.3.6. Testing for the presence of Saponins

A sample (I g) of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was closed with a stopper and vigorously shaken for about 30 seconds .The tube was then allowed to stand and observed for the formation of honeycombs whose possible appearance for at least an hour was taken as an evidence for the presence of Saponins.

2.2.3.7. Testing for the presence of Cyanogenic glycosides

A sample (3 g) of the powdered plant was placed in an Erlenmeyer flask and sufficient water was added to moisten the sample, followed by 1 ml of chloroform (to remove every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask , a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of Cyanogenic glycoside .

2.2.3.8. Testing for the presence of Anthraquinone glycoside

A sample (I g) of the powdered plant was boiled with 10ml of 5% ethanol for 5 minutes and then filtrated. The filtrate was washed with 10 ml hot ethanol followed by evaporation. Then 10 ml of the solution was extracted with 10 ml benzene, which was allowed to separate. Then the benzene layer was shaken with 10 ml of 10% ammonium hydroxide. The presence of Anthraquinone was indicated by pink and red colour.

2.2.4. Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about (10^8-10^9) colony forming units per ml using Mc Farland Scale. The suspension was stored in the refrigerator at 4°C until used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline in tubes and 0.02 ml volumes (one drop) of the appropriate dilutions were transferred by Transfer pipette adjustable volume (automatic microlitre pipette) onto the surface of dried nutrient agar plates.

The plates were allowed to stand for 2 hours at room temperature for the drops to dry, and then incubated at 37°C for 24 hours. After incubation the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units (C.F.U.)/per ml of suspension.

Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained so that suspensions with very close viable counts would be obtained.

2.2.5. In vitro Testing of extracts for antimicrobial activity

The cup-plate-agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications, to assess the antibacterial activity of the prepared extracts. Three ml of each of the five standardised bacterial stock suspensions ($IO^8 IO^9$ C.F.U. /ml) were thoroughly mixed with 300 ml of sterile melted nutrient agar which was maintained at 45°C.

20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates, which were divided to two halves, two cups in each half (10 mm in diameter) were cut using a sterile cork borer (No.4), each one of Petri-dishes halves was designed for one of the extracts.

The agar disks were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using Transfer pipette adjustable volume (automatic microliter pipette), and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Two replicates were carried out for each extract against each of the test organisms simultaneously; (positive) controls involving the addition of the respective solvents instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured, mean values were tabulated.

2.2.6. Clinical isolates

100 clinical isolates of *Staphylococcus aureus, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella spp* were collected randomly from the National Health Laboratory and Khartoum Teaching

Hospital. The clinical isolates were obtained from wounds and abscesses. They were purified by streaking on plates containing the appropriate selective and differential culture media. They were identified on the basis of the results of microscopical stain reaction (Gram stain), cultural characteristics and biochemical tests (Cruickshank et al, 1975).

2.2.7. Microscopical examination of aerobic bacterial isolates

All isolates were subjected to microscopical examination to study their stain properties (using the Gram's staining technique), the shapes and arrangements of their cells. In principle, the bacterial film is fixed and stained with a Triphenylmethane dye such as Crystal violet, in conjunction with Iodine solution, and subsequently treated with an organic solvent such as Alcohol. Bacteria which retain the dye are identified as Gram-positive and other varieties of bacteria which lose the dye are identified as Gram-negative. Decolourised organisms were rendered visible by the application of a counter stain of suitable colour e.g. Safranin red. Shapes and arrangements of the cells were also considered.

2.2.8. Biochemical tests

To ensure the correct identification of the Bacteria species Biochemical Tests were carried out depending on the metabolic differences between these species. The first tests were based on the ability of the Bacterial species to produce acidic and gaseous end-products, when presented with individual carbohydrate (glucose, lactose etc...) as the sole carbon source. The second tests were based on determining whether the Bacteria species produce particular end-product such as Indole, when grown in suitable culture media. The third tests were carried out to

determine whether the Bacteria species possess certain enzymic activities, that synonymous with Oxidase, Urease etc...

2.2.8.1. Fermentation tests

Bacteria are able to ferment carbohydrates (e.g. glucose, lactose, and sucrose), but this process varies from species to the other. In these tests sterile peptone water with 1 % of the sugar was used. Phenol red as indicator was added and Durham tube to detect if gases were produced. After 24 hours incubation, the colour of the inoculated medium changed from pink to yellow indicating fermentation and production of acids. The production of gas was detected by the presence of Air-bubbles in the apex of the inverted Durham tube (Salle, 1961).

2.2.8.2. Methyl-Red test

The Methyl-Red test was used to detect the ability of some bacteria to produce significant amounts of acidic substances due to fermentation of glucose. In this test Methyl-Red Voges-Proskauer medium was used. After inoculation, colour changed from yellow (pH 6.2) to red (pH 4.2) with acid production (Cruickshank et al.1975).

2.2.8.3. Voges - Proskauer test

The Voges - Proskauer test is based upon the production of Acetyl methyl carbinol, as a product of dextrose metabolism by certain bacteria. This substance is readily oxidized by atmospheric oxygen, in alkaline medium, giving diacetyl which, in turn, reacts with the amino acid Arginine in the inoculated medium to give a pink colour when alpha-naphthol is added (Cruickshank et al, 1975).

2.2.8.4. Citrate utilization test

The Citirate utilization test is based upon the ability of some organisms to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen. In this test the Koser citrate medium was inoculated with a 24 hours culture of the tested organism and then incubated at 37°C for 2-3 days. Then when examined the growth changed the colour of the incorporated bromothymol blue indicator from green to blue, due to citrate utilizaltion and production of alkali (Cruickshank *et al,* 1975).

2.2.8.5. Indole production test

The Indole production test is based on the ability of certain bacteria to oxidize the side chain of the amino acid Tryptophane with the production of Indole. In this test the peptone water was inoculated with the tested organism, incubated for two days and when Kovac's reagent was added the colour changed to red, indicating the presence of Indole (Cruickshank *et al,* 1975).

2.2.8.6. Hydrogen Sulphide (H2S) production test

The Hydrogen Sulphide (H_2S) production test is based upon the ability of some bacteria to produce H_2S from sulfur containing amino acids by reduction. H_2S was tested by suspending strips of filter paper impregnated with lead acetate above the culture. The presence of H_2S was demonstrated by its ability to form black insoluble lead sulfide when the inoculated peptone water was incubated at 37°C for 2-3 days (Cruickshank *et al,* 1975).

2.2.8.7. Kligler Iron agar (K.I.A.) test

The Kligler Iron agar (K.I.A.) test is based on the ability of some bacteria to ferment glucose and lactose and the production of H_2S gas. The tested organism was inoculated and incubated at 37 °C overnight. Changing of the colour of the slope or butt from pink to red indicated fermentation of glucose (alkaline reaction), and the changing of the colour from pink to yellow indicated fermentation of lactose (acid reaction), (Cheesbrough, 2000).

2.2.8.8. Catalase test

The Catalase test is based on the presence of the enzyme Catalase in the cells of certain bacteria, whish catalyses the release of oxygen from hydrogen peroxide. The test was carried out by addition of few drops of hydrogen peroxide on a 24 hours nutrient agar culture of the tested organism. The production of gas bubbles indicated a positive Catalase reaction (Salle, 1961).

2.2.8.9. Coagulase test

The Coagulase test is based on the presence of the enzyme coagulase in the cells of some bacteria. 1 in 10 dilution of citrated human plasma in saline was added to few drops of inoculated broth culture with the tested organism, and then the mixture was incubated at 37°C. The mixture was examined for coagulation after 1, 3 and 6 hours. The formation of clearly visible clots indicated a positive coagulase test (Cruickshank *el al,* 1975).

2.2.8.10. Oxidase test

The Oxidase test is based on the presence of the enzyme Oxidase in the cells of certain bacteria, which catalyzes the transport of electrons between electron donors in the bacteria and Tetramethyl-p-

phenylenediamine dihydrochloride. A freshly prepared oxidase reagent when added in a solid growth medium, a purple colour rapidly developed at the colonies of oxidase positive organisms (Cruickshank *et* al, 1975; Salle, 1961).

2.2.8.11. Urease test

The Urease test is based on the presence of the enzyme Urease in the cells of certain bacteria. Urease enzyme catalyzes the decomposition of urea and the production of ammonia. The test was carried out by growing the tested organism in presence of urea. Ammonia production was tested by means of suitable pH indicator e.g. phenol red. When colour changed from yellow to pink and ammonia produced a positive test was indicated (Salle, 1961).

2.2.8.12. Deoxyribonuclease (DNase) test

***The* Deoxyribonuclease (DNase) test is based on the ability of *Staphylococcus aureus* in producing the enzyme Deoxy ribonuclease (DNase). The tested organism was cultured on a medium which contained (DNA). After overnight incubation, the colonies were tested for DNase production by flooding the plate with a (IN) HCl acid solution. The acid precipitated unhydrolysed DNA. DNase producing colonies were therefore surrounded by clear areas indicating DNA hydrolysis (Cheesbrough, 1996).**

2.2.9. Testing the susceptibility of clinical isolates to herbal extracts

The standard cup plate agar diffusion technique was used to determine the susceptibility of the clinical strains to the various extracts which showed activity against standard bacterial organisms.

2.2.9.1. Determination of minimum inhibitory concentration (MIC) by agar plate dilution method

The principle of the agar plate dilution method is testing the inhibition of the growth of the seeded bacteria on the surface of the agar by the incorporated plant extracts. Plates were prepared by adding a series of increasing concentrations of the plant extract to the media, in the following order: 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml. The bottom of each plate was marked into five segments to test the standard bacteria. The organism to be tested was grown in broth over-night, and then diluted in broth to contain about 10 cells per ml. A loop-ful of the diluted culture is spotted with standard-loop that delivers 0.01 ml onto the surface of each segment and then incubated at 37°C for 18 hours.

2.2.9.2. Reading of (MIC) plates

The Minimum Inhibitory Concentration (MIC) is the least concentration of any antimicrobial agent that completely inhibits bacterial growth. Results are reported as the MIC in mg/ml of crude extract.

2.2.10. Antibacterial activity of commonly used antibiotics against standard organisms

Four Antibiotics (Benzylpenicillin, Gentamycin, Ceftriaxone and Erythromycin) were prepared in suspensions of four concentrations (40, 20, 10 and 5 µg/ml) using sterile distilled water; to evaluate their antibacterial activity against the tested standard bacterial organisms, using the cup plate agar diffusion method described above.

Removed cups were filled with 0.1 ml samples of each of the four concentrations of the reference drugs using Transfer pipette adjustable volume automatic microtitre pipette, and allowed to diffuse at room

temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Two replicates were carried out for each concentration against each of the test organisms simultaneously. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Chapter Three

Results

3. Results

3.1. Identification of Clinical Bacterial Organisms

Standard and clinical bacterial organisms were used to evaluate their susceptibility to four standard chemotherapeutic agents. As shown in table 5, the standard bacterial organisms were obtained from the National Collection of Type Culture (NCTC), Colindale, England and the American Type Culture Collection (ATCC), Rockville, Maryland, USA.

The clinical bacterial organisms were isolated from swaps taken from wounds and abscesses. A total of 100 isolates were randomly taken and they were found to consist of 20, 20, 18, 17, 18 and 7 of ***Escherichia coli***; ***Pseudomonas aeruginosa***; ***Proteus vulgaris; Klebseilla sp.; Staphylococcus aureus***; and Methicillin-resistant ***Staphylococcus aureus*** (MRSA) isolates respectively. These bacterial species were identified adopting the standard methods.

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3.1.1. Identification of Escherichia coli

A. Cultural characteristics

After 24 hours incubation of the cultured Mac Conkey's agar medium, red colonies were observed, this was attributed to lactose fermentation. This Cultural characteristic is synonyms with ***Escherichia coli***. However, to confirm this result, more tests were carried out.

B. Microscopical examination

The bacterial isolates were subjected to microscopical examination using the Gram's staining technique (Cruickshank et al, 1975), Gram negative rods were seen. This result is a further confirmation that the bacterium earlier identified was ***Escherichia coli***. For conclusiveness biochemical reaction tests were carried out and the results are given below.

C. Biochemical reactions

The bacterial isolates were subjected to biochemical reaction tests. The bacterium was identified as ***Escherichia coli***, hence confirming the results obtained adopting the above two tests. The bacterium identification was based on the following results:

1. The fermentation test, described in section 2.2.4.3.1, showed that all isolates fermented lactose, manitol and glucose and produced acid and gas, and most of them fermented sucrose and produced acid and gas (Salle, 1961).
2. The Methyl Red positive test, described in section 2.2.4.3.1, gave positive results for all isolates which fermented glucose and produced acid (Cruickshank et al, 1975).
3. The Voges Proskauer test, described in section 2.2.4.3.3, gave negative results for all isolates that did not metabolize dextrose, and Acetyl methyl carbinol was not produced (Cruickshank et al, 1975).
4. Indole production test, described in section 2.2.4.3.3, showed that all isolates oxidised the side chain of the amino acid Tryptophane and produced Indole (Cruickshank et al, 1975).

5. **Citrate utilization test, described in section 2.2.4.3.4, showed that none of the tested isolates utilized citrate (Cruickshank et al, 1975).**
6. **The Oxidase test, as described in section 2.2.4.3.10, no purple colour was developed, indicating negative results (Cruickshank et al, 1975; Salle, 1961).**
7. **The Urease test, as described in section 2.2.4.3.11 the yellow colour didn't change to pink indicating that there was no ammonia produced, which was a negative result (Salle, 1961)**
8. **Kligler Iron agar (K.I.A.) test, described in section 2.2.4.3.7, showed that all isolates did not change the yellow colour of K.I.A. neither in slope nor in butt, with the absence of H_2S production, however all the tested isolates produced gas (Cheesbrough, 2000).**

3.1.2. Identification of Proteus vulgaris

A. Cultural characteristics

After 24 hours incubation of the cultured Nutrient agar, a fishy smell was detected and swarming appearance was seen. Whereas on the cultured Mac Conkey's agar medium, pale coloured colonies were observed indicative of non-lactose fermentation. These Cultural characteristics are synonyms with *Proteus vulgaris*. However, to confirm this result, more tests were carried out.

B. Microscopical examination

The bacterial isolates were subjected to microscopical examination using the Gram's staining technique (Cruickshank et al, 1975), where Gram negative rods were seen. This further confirmed that the

bacterium was *Proteus vulgaris*. However, biochemical reaction tests were carried out to corroborate these results.

C. Biochemical reactions

The bacterial isolates were subjected to biochemical reaction tests. The bacterium was identified as *Proteus vulgaris,* hence confirming the results obtained from the above described tests. The bacterium identification was based on the following results:

1. **The fermentation test described in section 2.2.4.3.1 showed that not all isolates fermented lactose, and most of them did not ferment mannitol or sucrose and none produced acid; however, some of them formed gas (Salle, 1961).**
2. **The Methyl Red positive test described in section 2.2.4.3.1 gave positive results for all the isolates; they fermented glucose and produced acid (Cruickshank et al, 1975).**
3. **The Voges Proskauer test described in section 2.2.4.3.3 gave negative results for all the tested isolates, they didn't metabolise dextrose and Acetyl methyl carbinol was not produced (Cruickshank et al, 1975).**
4. **The Indole production test described in section 2.2.4.3.3 showed that all the isolates oxidised the side chain of the amino acid Tryptophane and Indole was produced (Cruickshank et al, 1975).**
5. **The Citirate utilization test described in section 2.2.4.3.4 showed that the isolates utilized citrate (Cruickshank et al, 1975).**
6. **The Oxidase test, as described in section 2.2.4.3.10, no purple colour was developed indicating negative results (Cruickshank et al, 1975; Salle, 1961).**

7. The Urease test, as described in section 2.2.4.3.11 the yellow colour changed to pink as a results of ammonia production indicating positive results (Salle, 1961; Cruickshank et al, 1975).
8. The Kligler Iron agar (K.I.A.) test described in section 2.2.4.3.7 showed that all the isolates changed the yellow colour of K.I.A. to red in slope and no change of colour in the butt, H_2S was produced, but no gas was produced (Cheesbrough, 2000).

3.1.3. Identification of Pseudomonas aeruginosa

A. Cultural characteristics

After 24 hours incubation of the cultured Nutrient agar, blue-green pigments was produced and diffused in the surrounding media. These Cultural characteristics are synonyms with ***Pseudomonas aeruginosa***. However, to confirm this result, more tests were carried out.

B. Microscopical examination

The bacterial isolates were subjected to microscopical examination using the Gram's staining technique, and Gram negative rods were seen. This further confirmed that the bacterium was ***Pseudomonas aeruginosa***. However, for more confirmation, biochemical reaction tests were carried out.

C. Biochemical reactions

The bacterial isolates were subjected to biochemical reaction tests. The bacterium was identified *as* ***Pseudomonas aeruginosa*** hence confirming the results obtained from the above two tests. The bacterium identification was based on the following results:

1. **The fermentation test described in section 2.2.4.3.1 showed that all isolates did not ferment lactose, and most of them fermented glucose and did not ferment sucrose (Salle, 1961).**
2. **The Methyl Red positive test described in section 2.2.4.3.1 gave negative results for all isolates which didn't ferment glucose and didn't produce acid Cruickshank et al, 1975).**
3. **The Voges Proskauer test described in section 2.2.4.3.3 gave negative results for all isolates which didn't metabolise dextrose and Acetyl methyl carbinol was not produced Cruickshank et al, 1975).**
4. **Indole production test described in section 2.2.4.3.3 showed that all isolates didn't oxidise the side chain of the amino acid Tryptophane and didn't produce Indole Cruickshank et al, 1975).**
5. **The Citrate utilization test described in section 2.2.4.3.4 showed that the isolates didn't utilize citrate indicating negative results (Cruickshasnk et al, 1975).**
6. **The Oxidase test, as described in section 2.2.4.3.10, a purple colour was developed indicating postive results (Cruickshank et al, 1975; Salle, 1961).**
7. **The Urease test, as described in section 2.2.4.3.11 the yellow colour changed to pink as a results of ammonia production indicating positive results (Salle, 1961; Cruickshank et al, 1975).**
8. **The Kligler Iron agar (K.I.A.) test described in section 2.2.4.3.7 showed that all isolates changed the yellow colour of K.I.A. to red in slope and most of the isolates changed of colour to red in the butt, no H2S or gas was produced (Cheesbrough, 2000).**

9. The Oxidase test described in section 2.1.8.10 showed that when oxidase reagent was added in the solid growth medium, a purple colour was rapidly developed indicating positive oxidase production (Cruickshank et al, 1975; Salle, 1961).

3.1.4. Identification of Stapliylococcus aureus

A. Cultural characteristics

After 24 hours incubation of the cultured Nutrient agar, golden yellow colonies were observed. On Mannitol salt agar, the colour of medium changed from red to yellow These Cultural characteristics are synonyms with ***Stapliylococcus aureus***. However, to confirm this result, additional tests were carried out.

B. Microscopical examination

The bacterial isolates were subjected to microscopical examination using the Gram's staining technique, and Gram positive cocci arranged in grape-like clusters were seen. This further confirmed that the bacterium was ***Stapliylococcus aureus***. However, for more confirmation, various biochemical reaction tests were carried out (Cruickshank et al, 1975).

C. Biochemical reactions

The bacterial isolates were subjected to biochemical reaction tests. The bacterium was identified as ***Stapliylococcus aureus*** hence confirming the results obtained from the above two tests. The bacterium identification was based on the following results:

1. The fermentation test described in section 2.2.4.3.1 showed that all isolates fermented lactose and produced acid and no gas, and they

fermented sucrose and mannitol and produced acid. Most of the isolates fermented glucose and produced acid (Salle, 1961).

2. The Catalase test described in section 2.2.4.3.8 showed that all isolates were catalase positive. They catalysed the release of oxygen from hydrogen peroxide, where gas bubbles were observed (Cruickshank et al, 1975).
3. The Coagulase test described in section 2.2.9.9 showed that all isolates were coagulase positive; visible clots were formed indicating the presence of the enzyme coagulase (Cruickshank et al, 1975).
4. The Deoxyribonuclease (DNase) test described in section 2.2.9.12 showed that all isolates were DNase positive. It was observed that DNase producing colonies were surrounded by clear areas indicating DNA hydrolysis because of adding HCL acid to the cultured medium that contained DNA (Cheesbrough, 1996).

3.1.5. Identification of Klebsiella sp

A. Cultural characteristics

After 24 hours incubation of the cultured Nutrient agar, golden yellow colonies were observed. On Mannitol salt agar, the colour of medium changed from red to yellow. These Cultural characteristics are synonyms with ***Klebsella sp.*** However, to confirm this result, more tests were carried out.

B. Microscopical examination

The bacterial isolates were subjected to microscopical examination using the Gram's staining technique, and Gram negative capsulated rods were seen. This further confirmed that the bacterium was ***Klebsella sp***.

However, for more confirmation, biochemical reaction tests were carried out (Cruickshank et al, 1975).

C. Biochemical reactions

The bacterial isolates were subjected to biochemical reaction tests. The bacterium was identified as *Klebsella sp,* hence confirming the results obtained from the above two tests. The bacterium identification was based on the following results:

1. **The fermentation test described in section 2.2.4.3.1 showed that all isolates fermented lactose, sucrose and manitol and produced gas (Salle, 1961).**
2. **The Citrate utilization test described in section 2.2.4.3.4 showed that the isolates utilized citrate indicating posative results (Cruickshasnk et al, 1975).**
3. **The Oxidase test, as described in section 2.2.4.3.10, no purple colour was developed indicating negative results (Cruickshank et al, 1975; Salle, 1961).**
4. **The Urease test, as described in section 2.2.4.3.11 the yellow colour changed to pink as a results of ammonia production indicating positive results (Salle, 1961; Cruickshank et al, 1975).**
5. **The Catalase test described in section 2.2.4.3.8 gas bubbles were seen due to oxygen production indicating posative results. (Salle, 1961).**

The results of the clinical bacterial organisms' identification were shown in table 6.

3.2. Medicinal plants phytochemical screening

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described in section 2.2.3 which were based on methods adopted by various authors (Vogel, 1958; Kapoor et al., 1969; Rizk and Bashir, 1980; Fadeyi et al., 1989; Odebiyi and Sofowora, 1990). The medicinal plants extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids. The results obtained are discussed and tabulated (Table 8) below.

Phytochemical analysis showed that:

1. ***Piper cubeba*** **extract contains Tannins, Flavonoids, Cardiac glycoside, phenolic glycoside, and Alkaloids.**
2. ***Anogussus leiocapus*** **contains Tannins, Saponins, phenolic glycoside, Alkaloids and only traces of Anthraquinone glycoside and Cyanogenic glycosides.**
3. ***Biota orientalis*** **extract contains Saponins, Tannins, Alkaloids and only traces of Cardic glycoside.**
4. ***Punica granatum*** **(Bark) extract contains Saponins, Tannins, Anthraquinone glycoside, Alkaloids and only traces of Cardic glycoside, phenolic glycoside.**
5. ***Punica granatum*** **(Wood) extract contains Saponins, Tannins, Anthraquinone glycoside, Alkaloids, Cardic glycoside and phenolic glycoside.**

6. ***Calendula arvensis*** **extract contains Tannins, Flavonoids and only traces of Anthraquinone glycoside.**
7. ***Salvia officinalis*** **extract contains Tannins, Anthraquinone glycoside, Cardic glycoside and Alkaloids.**
8. ***Nauclia latifolia*** **extract contains Flavonoids, phenolic glycoside, Alkaloids.**
9. ***Cissus petiolata*** **extract contains Saponins, Flavonoids, phenolic glycoside, Cyanogenic glycosides.**
10. ***Petasties hybriduss L.*** **extract contains Saponins, Tannins, Flavonoids, phenolic glycoside, Alkaloids.**
11. ***Acorus calamus*** **extract contains Saponins, Flavonoids, Cardic glycoside, Alkaloids.**
12. ***Ferula asafoetida*** **extract contains Flavonoids only traces of phenolic glycoside, Cyanogenic glycosides.**
13. ***Moringa peregrina*** **extract contains Saponins, Flavonoids, Cyanogenic glycosides, Alkaloids.**

3.3. Susceptibility of standard bacteria to standard chemotherapeutic agents

The antimicrobial activity of the four conventional chemotherapeutic agents against five standard bacterial organisms was determined. The chemotherapeutic agents which are currently in use worldwide are Benzylepenicillin, Erythromycin, Gentamycin and Ceftriaxone. Varying concentrations (40, 20, 10 and 5 µg/ml) of these agents were used in the investigations. The standard bacterial organisms tested were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginos,*

Table 8: Chemical constituent of the 13 medicinal plants

Latin name	Arabic name	Yield% of MeOH extract	Phytochemical groups							
			1	2	3	4	5	6	7	8
Piper cubeba	حب العروس	2.13	-	+	-	+	+	+	-	+
Anogussus leiocapus	الصحب	1.3	+	+	±	-	-	+	±	+
Blota (Thuja) orientalis	العفصة	0.74	+	+	-	-	±	-	-	+
Punica granatum (Bark)	الرمان	3.8	+	+	+	-	±	±	-	+
Punica granatum (Wood)	الرمان	2.7	+	+	+	-	+	+	-	+
Calendula arvensis	الحنوه	2.45	-	+	±	+	-	-	-	-
Salvia officinalis	مريمية	0.84	-	+	+	-	+	-	-	+
Nauclia latifolia	كرمدودة	3.12	-	-	-	+	-	+	-	+
Cissus petiolata	عرق الحجر	3.14	+	-	-	+	-	+	+	-
Petasties hybriduss	فكهت	2.22	+	+	-	+	-	+	-	+
Acorus calamus	الذر يره	0.24	+	-	-	+	+	-	-	+
Ferula asafoetida	الحلتيت	1.14	-	-	-	+	-	±	±	-
Moringa perigrina	الراواق	5.34	+	-	-	+	-	-	+	+

1: Saponins, 2: Tannins ; 3: Anthraquinone glycoside ; 4: Flavonoids ; 5 : Cardic glycoside ; 6 : Phenolic gycoside ; 7 : Cyanogenic glycosides ; 8 : Alkaloids.(+) : positive ; (-) : negative ; (±) : Traces

Klebsiella sp **and** ***Bacillus subtilis*** **(Table 5). The potency of the four antibiotics was assessed by the presence or absence of inhibition zone and zone diameter, respectively. The results in terms of averages of inhibition zones are given in Table 9. The commonly used terms: resistant and sensitive were adopted in this study to interpret the results obtained.**

The aim of this part of the study was to compare the antimicrobial activity of the four standard chemotherapeutic agents which was determined in this part of the study to the antimicrobial activity of the same agents and the 12 medicinal plants extracts when tested against clinical bacterial organisms (section 3.4).

The results (Tables 9 and 10 and Figures 1 and 2) show that the antibacterial efficacy of the four standard chemotherapeutic agents follows the ascending order: Benzylepenicillin, Erythromycin > Gentamycin > Ceftriaxone. It was found that there was significant differences between the four standard chemotherapeutic agents in terms of their antibacterial effectiveness against the test standard bacteria. From Table 9 it could be seen that compared to the other agents, Benzylepenicillin is the least effective, it showed no antibacterial effect against all test bacterial strains. The results also, showed that the Erythromycin when tested at the four concentrations used, showed low antibacterial activity. It was active only against *Staphylococcus aureus*. Whereas, Ceftriaxone showed the highest antibacterial effectiveness, at the four tested concentrations, it inhibited the growth of all test bacterial organisms at varying levels, except *Klebsiella sp*. Gentamycin was the second effective antibacterial agent; at all the concentrations, it inhibited the growth of all test bacterial organisms except *Pseudomonas aeruginosa* when tested at the two lowest concentrations (10 and 5 µg/ml). In general, it was observed that the inhibition level varies according to the bacterial species and the antibiotic and its concentration.

The results in Table 9 and 10 and Figures 1- 5 show that the susceptibility of the test bacteria follows the ascending order: *Klebsiella sp>*

Pseudomonas aeruginosa **>** ***Bacillus subtilis*** **>** ***Escherichia coli*** **>** ***Staphylococcus aureus*****. The results also, showed significant differences between the five standard bacterial organisms in terms of their susceptibility to the standard chemotherapeutic agents.**

Table 9: Antibacterial Activity of Chemotherapeutic Agents against Standard Bacterial Organisims

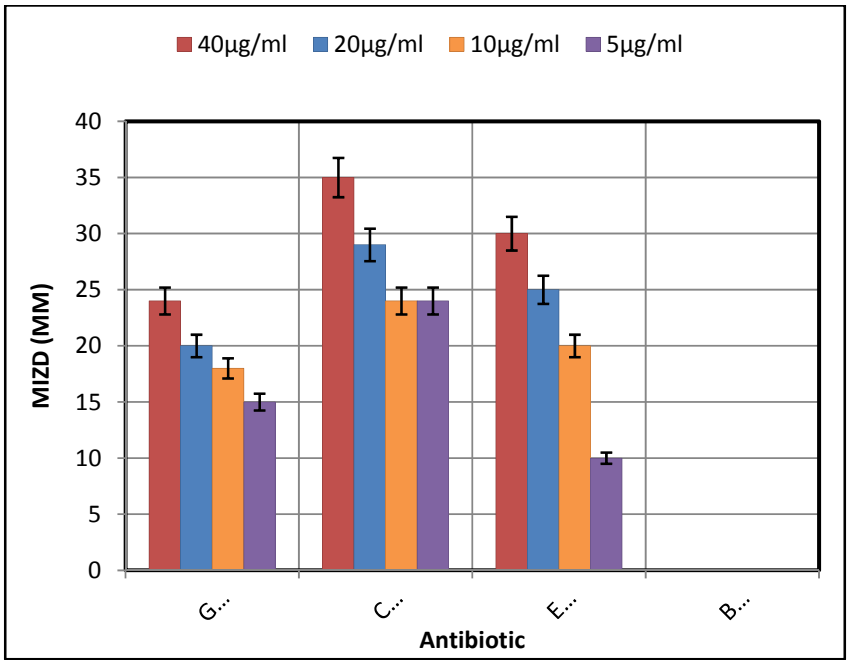
Chemotherapeutic Agents	***Concentration µg/ml***	***MIZD(mm)***				
		Ec	Sa	Ps a	Kl	Bs
Benzylepenicillin	40	0	0	0	0	0
	20	0	0	0	0	0
	10	0	0	0	0	0
	5	0	0	0	0	0
Erythromycin	40	0	30	0	0	35
	20	0	25	0	0	30
	10	0	20	0	0	25
	5	0	10	0	0	40
Gentamycin	40	25	24	15	25	29
	20	20	20	15	25	25
	10	15	18	0	25	22
	5	15	15	0	25	20
Ceftriaxone	40	36	35	32	0	19
	20	30	29	30	0	17
	10	25	24	22	0	13
	5	23	24	20	0	12

Standard Organisms used: Ec: *Escherichia coli*; Sa*: Staphylococcus aureus*; Ps a: *Pseudomonas aeruginosa*; Kl*: Klebsella sp*; Bs: *Bacillus subtilis.*
MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: Intermediate; < 14: Resistant; 0: No inhibition).

According to the results shown in Table 9, the least susceptible bacterium was ***Klebsella sp*****. it has shown susceptibility to none of the tested antibiotics except Gentamycin.** ***Bacillus subtilis*** **showed susceptibility to Gentamycin at all tested concentrations (40, 20, 10 and 5 µg/ml) and to Ceftriaxone at the two highest concentrations (40 and 20 µg/ml). The results also, showed that** ***Staphylococcus aureus*** **was the**

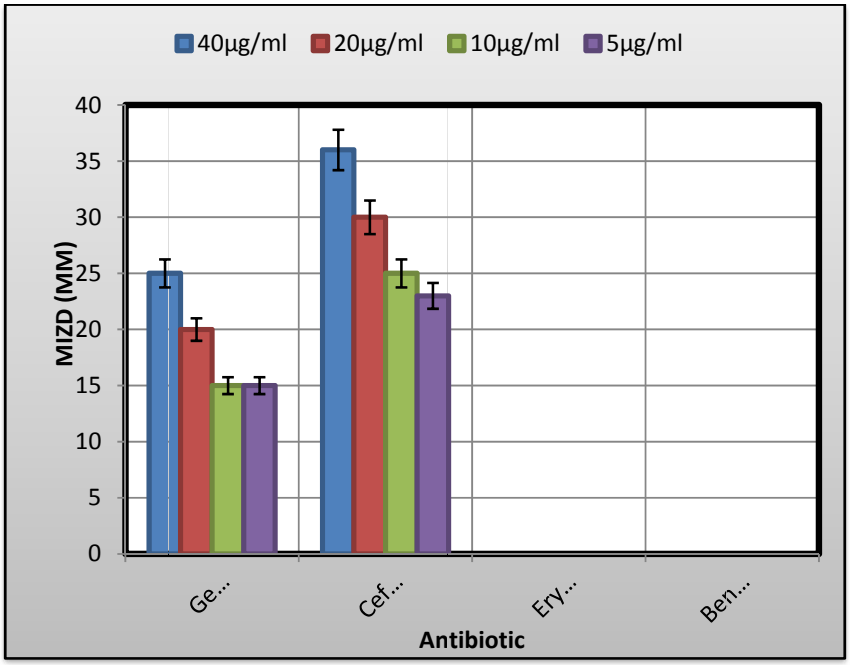
most susceptible to antibiotics; it has shown susceptibility to all tested antibiotics except Benzylepenicillin. Both ***Bacillus subtilis*** and ***Escherichia coli*** showed moderate susceptibility to antibiotics, they showed susceptibility to just two of the tested antibiotics (Gentamycin and Ceftriaxone). The differences in the level of susceptibility are most likely due to the mode and site of action of the chemotherapeutic agents.

Figure 1: Antibacterial Activity of Chemotherapeutic Agents against Standard *Staphylococcus aureus*



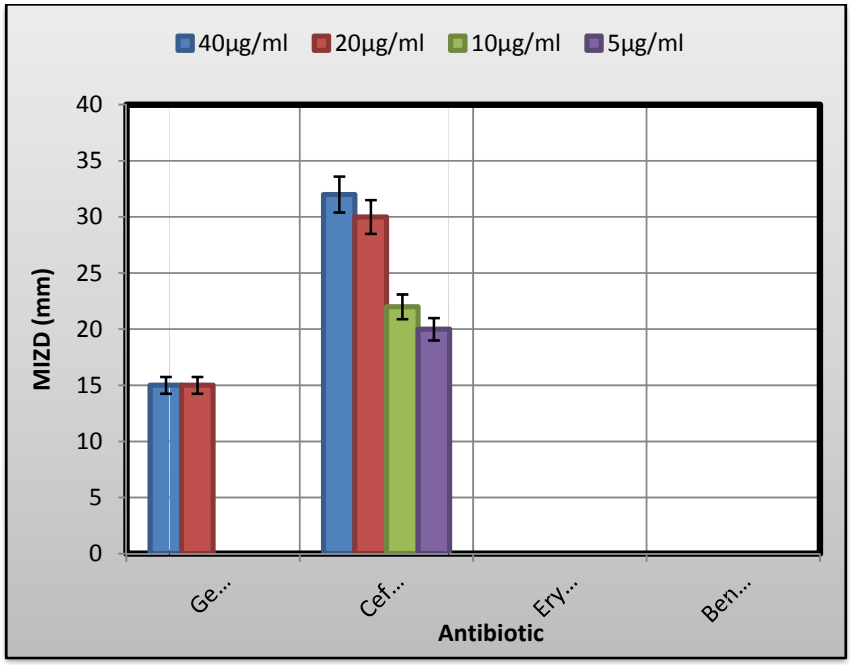
MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: Intermediate; < 14: Resistant; 0: No inhibition).

Figure 2: Antibacterial Activity of Chemotherapeutic Agents against Standard *Escherichia coli*



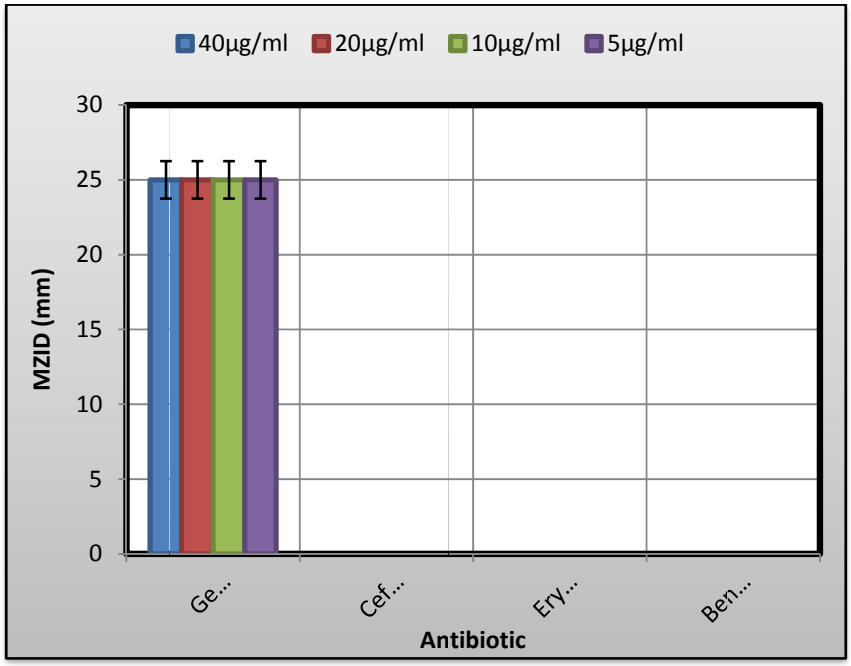
MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: Intermediate; < 14: Resistant; 0 : No inhibition).

Figure 3: Antibacterial Activity of Chemotherapeutic Agents against Standard *Pseudomonas aeruginosa*



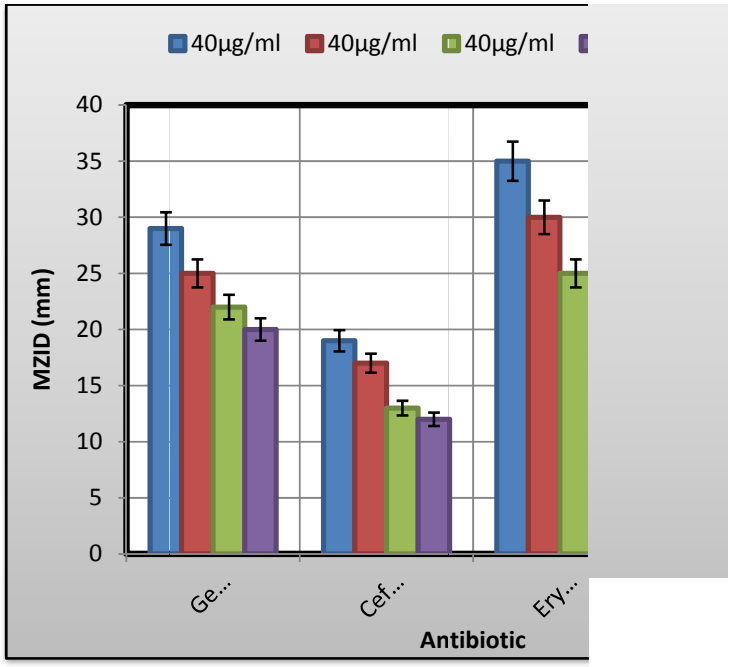
MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: Intermediate; < 14: Resistant; 0 : No inhibition)

Figure 4: Antibacterial Activity of Chemotherapeutic Agents against Standard *Klebsiella sp*



MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: Intermediate; < 14: Resistant; 0 : No inhibition).

Figure 5: Antibacterial Activity of Chemotherapeutic A
Standard *Bacillus subtilis*



MIZD (mm): Mean Inhibition Zone Diameter (>15mm: Sensitive; 14-15: I
< 14: Resistant; 0 : No inhibition).

3.4. Susceptibility of standard bacter Medicinal Plants Extract

The antimicrobial activity of 13 medicinal plants extra standard bacterial organisms (***Escherichia coli; Staphyl Pseudomonas aeruginosa; Klebsella sp; Bacillus subtilis*** The five standard organisms were obtained from the Na of Type Culture (NCTC), except the ***Bacillus subtilis*** whi from the American Type Culture Collection (ATCC) (section 3.3, four standard chemotherapeutic agent assessed by the presence or absence of inhibition diameter, respectively as given in Table 9. Benzene,